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ERRATA

Volume 27, Number 2, October, 1940

"The Peroxide Effect in the Addition of Reagents to Unsaturated Compounds and in Rearrangement Reactions," by Frank R. Mayo and Cheves Walling:

Page 352, line 8: Substitute "rearrangement" for "substitution."

Page 354, line 25: Substitute "toward" for "to."

Page 354, footnote 3, line 6: Substitute "(99)" for "(112)".

Page 369, last line: Substitute "insoluble" for "soluble."

Page 372, section 5, line 18: Substitute "bromide ion" for "proton."

Page 400, equation 31: Substitute " $\text{CH}_2=\text{CHCHCH}_3$ " for the first formula ($\text{CH}_2\text{C}=\text{HCHCH}_3$).

In a recent article by E. A. Doisy, S. B. Binkley, and S. A. Thayer (Chem. Rev., **28**, 477 (1941)) the term "darco" was used to describe an adsorbent used in the purification of vitamin K. Attention should have been called to the fact that "Darco" has been registered as a trade mark in the U. S. Patent Office (126,469) and in many other countries to identify activated carbon products sold and distributed by the Darco Corporation, Wilmington, Delaware.

INTERPRETATION OF THE POLAROGRAPHIC WAVES OF COMPLEX METAL IONS

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Received October 2, 1940

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I. INTRODUCTION

The general principles and practical applications of polarographic analysis with the dropping mercury electrode have been reviewed by Heyrovsky (4), Hohn (7), Semerano (16), Stackelberg (18), and Kolthoff and Lingane (9), among others, and Heyrovsky and Klumpar (6) have compiled a complete bibliography of this field up to 1939. Although the utility of the polarographic method as a practical analytical technique is becoming well known, the fact that the method can also be applied advantageously to more abstract problems in inorganic chemistry and electrochemistry has been less generally recognized. The purpose of this paper is to discuss the application of the polarographic technique to the study of complex metal ions in aqueous solution, with emphasis on the fundamental principles that are involved in the interpretation of the "polarographic waves."

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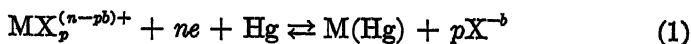
The study of complex metal ions by the polarographic method rests on the fact that the reduction potentials of metal ions at the dropping electrode are shifted (usually to a more negative value) by complex formation, and by measuring this shift as a function of the concentration of the complex-forming substance both the formula and the dissociation constant of the complex can be determined. *It should be emphasized that this information can only be obtained when the reduction or oxidation of the metal ion complex is reversible at the dropping electrode.* Therefore it is essential to establish the reversibility of the electrode reaction in any given case, before attempting to draw quantitative conclusions from the experimental data. For this reason, the following discussion is devoted chiefly to the derivation, and illustration by typical experimental examples, of relations that can be used for testing the reversibility at the dropping electrode of the various types of electrode reactions of complex metal ions. The fundamental principles on which these relations are based were first recognized by Heyrovsky and Ilkovič (5) in 1935.

The polarographic literature contains numerous papers dealing with the behavior of complex metal ions, chiefly from a practical analytical viewpoint (4, 6, 7, 9), but most of these studies were published prior to 1935 before the thermodynamic significance of the half-wave potential was appreciated. The older data have generally been reported in terms of the arbitrary "45° tangent reduction potential," and, since in most instances complete polarograms and other essential information are lacking, a satisfactory interpretation of this material from the modern viewpoint is virtually impossible and will not be attempted here. A complete review of the papers in this field has been given by Kolthoff and Lingane (9).

The various possible types of electrode reactions that involve complex metal ions may be divided into the following categories: (1) reduction to the metallic state with the formation of an amalgam on the surface of the dropping electrode; (2) reduction or oxidation from one ionic oxidation state to another; (3) stepwise reduction, resulting in the appearance of two or more separate waves; and (4) reduction of incompletely dissociated metal salts. The characteristics of the waves of each of these cases, with experimental examples, are discussed in the following pages.

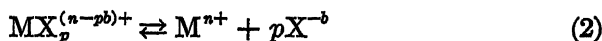
II. REDUCTION TO THE METALLIC STATE

The reduction to the metallic state (amalgam) of a complex ion of a metal that is soluble in mercury may be represented by

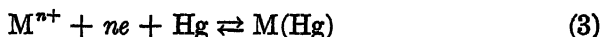


where $M(Hg)$ represents the amalgam formed on the surface of the dropping electrode, and X^{-b} is the complex-forming substance. It is convenient,

although not strictly necessary, to regard this net reaction as the sum of the two partial reactions



and



where M^{n+} symbolizes the "simple" or hydrated ions of the metal. The assumption of these two partial reactions is merely an artifice which assists in clarifying the thermodynamic relations that are involved, and it is not intended to indicate an actual kinetic mechanism. The following derivations are independent of the kinetics of the electrode reaction, provided that all the intermediate steps are very rapid compared to diffusion rates.

The fundamental assumption used to derive the equation of a polarographic wave is that the rates of all of the possible intermediate steps in the electrode reaction are so much more rapid than diffusion rates that the dropping electrode is subject only to concentration polarization (5). When this condition is fulfilled the electrode reaction will proceed reversibly, and the current at any point on the wave will be diffusion controlled and can be expressed in terms of the diffusion processes around the dropping electrode.

If the foregoing reactions are rapid and reversible at the dropping electrode, then the potential of the latter at any point on the wave should be given by

$$E_{d.e.} = E_a^0 - \frac{RT}{nF_v} \ln \frac{C_a^0 f_a}{a_{Hg} C_M^0 f_M} \quad (4)$$

In this equation, C_a^0 is the concentration of the amalgam formed on the surface of the mercury drops, C_M^0 is the concentration of the simple metal ions at the electrode surface, f_a and f_M are the corresponding activity coefficients, and a_{Hg} is the activity of the mercury on the surface of the dropping electrode. E_a^0 is the standard potential of the metal amalgam, which may be defined as the E.M.F. of the cell



when the quantity $C_a^0 f_a / a_{Hg} C_M^0 f_M$ is equal to 1. Since the amalgams formed on the surface of the dropping electrode are very dilute, a_{Hg} is virtually the same as the activity of pure mercury and may be regarded as a constant. Hence equation 4 may be written

$$E_{d.e.} = \epsilon - \frac{RT}{nF_v} \ln \frac{C_a^0 f_a}{C_M^0 f_M} \quad (5)$$

where the constant ϵ is equal to

$$E_a^0 + \frac{RT}{nF_v} \ln a_{Hg} \quad (6)$$

If the dissociation of the complex ion is sufficiently rapid so that equilibrium with respect to reaction 2 is practically maintained at the electrode surface, then C_M^0 can be replaced by

$$C_M^0 = K_o \frac{C_{MX}^0 f_{MX}}{(C_X^0)^p f_X^p f_M}$$

where K_o is the dissociation constant of the metal ion complex, C_{MX}^0 and C_X^0 are, respectively, the concentrations at the electrode surface of the complex metal ion and the complex-forming substance, X^{-b} , and the f 's are activity coefficients. Therefore equation 5 becomes

$$E_{a.s.} = \epsilon + \frac{RT}{nF_v} \ln \frac{K_o f_{MX}}{f_a f_X^p} - \frac{RT}{nF_v} \ln \frac{C_a^0 (C_X^0)^p}{C_{MX}^0} \quad (7)$$

The values of $E_{a.s.}$, and the various concentrations at the electrode surface, vary periodically during the life of each mercury drop, owing to the periodic change in the electrode area as each drop grows and falls (8, 9). Unless stated to the contrary, the *average* values of these quantities during the life of each drop will be understood in the following discussion.

We shall also assume that sufficient supporting electrolyte is present to eliminate the "migration current" (9, 13), so that diffusion is the sole process by which the reaction components are transferred between the electrode surface and the body of the solution.

A. Equation of the wave

In order to express the various concentrations at the electrode surface as a function of the current, we employ the fact that the diffusion layer around the mercury drops is so thin (*ca.* 0.05 mm.) that the concentration gradients are practically linear. If this assumption is valid, the concentration of the complex metal ions at the electrode surface will decrease in direct proportion to the increase in current along the wave, and we have

$$i = k_o (C_{MX} - C_{MX}^0) \quad (8)$$

or

$$C_{MX}^0 = C_{MX} - \frac{i}{k_o} \quad (9)$$

where C_{MX} and C_{MX}^0 are, respectively, concentrations in the body of the solution and at the electrode surface, and i is the *average* current (micro-

amperes) during the life of each mercury drop at any given value of $E_{d.e.}$. The constant k_0 is defined by the Ilkovič equation (8) and at 25°C., and when C_{MX} is expressed in millimoles per liter, it is given by (8, 9, 13)

$$k_0 = 605nD_{MX}^{1/2}m^{2/3}t^{1/6} \quad (10)$$

where D_{MX} is the diffusion coefficient of the metal ion complex ($\text{cm.}^2 \text{sec.}^{-1}$), t is the drop time (sec.), and m is the rate of flow of mercury from the dropping electrode (mg. sec.^{-1}). When the diffusion current, i_d , is reached, C_{MX}^0 has decreased to a constant minimal value which is negligibly small compared to C_{MX} ; hence from equation 8 we have

$$i_d = k_0 C_{MX} \quad (11)$$

which is the well-known linear relation between the diffusion current and concentration on which quantitative polarographic analysis is based (4, 7, 9, 13). In view of equation 11, equation 9 may be written

$$C_{MX}^0 = \frac{i_d - i}{k_0} \quad (12)$$

It will be evident that the concentration of the amalgam formed at any point on the wave must be directly proportional to the current,—

$$C_a^0 = k' i = \frac{i}{k_a} \quad (13)$$

where k_a is defined by an equation identical with equation 10, except that the square root of the diffusion coefficient of the metal in the amalgam, $D_a^{1/2}$, is employed.

Since the complex-forming substance X^{-b} is a product of the electrode reaction, its concentration at the electrode surface increases with increasing current. We have

$$C_X^0 = C_X + p \frac{i}{k_X} \quad (14)$$

where k_X is proportional to the square root of the diffusion coefficient of X^{-b} (equation 10), and C_X is the concentration of X^{-b} in the body of the solution. However, for the sake of simplicity, and since this is usually the case, we shall assume that the solution originally contains an excess of X^{-b} at a concentration that is relatively large compared to the concentration of the complex metal ion. When this condition is fulfilled, the quantity pi/k_X in equation 14 will be negligibly small, and C_X^0 can be regarded as virtually a constant and equal to C_X .

When the foregoing relations are substituted into equation 7, we obtain for the equation of the wave at 25°C., with an excess of the complex-forming

substance present in the solution,

$$E_{d.s.} = E_{1/2} - \frac{0.0591}{n} \log \frac{i}{i_d - i} \quad (15)$$

The half-wave potential, $E_{1/2}$, is given by

$$E_{1/2} = \epsilon + \frac{0.0591}{n} \log \frac{K_o f_{MX} k_a}{f_a k_o} - p \frac{0.0591}{n} \log C_X f_X \quad (16)$$

Equation 15 is valuable for testing the reversibility of the reduction, as will be shown later by several experimental examples.

It will be noted that the concentration of the metal ion complex does not enter into the foregoing expression for its half-wave potential; hence $E_{1/2}$ should be constant and independent of the concentration of the complex metal ion. The fundamental reason for this is that the ratio $i/(i_d - i)$ is always equal to 1 at the midpoint of the wave, where $i = i_d/2$, and hence the log term in equation 15 becomes zero at this point regardless of the concentration of the complex metal ion. As a matter of fact, the half-wave potential exhibits this important property in any type of reversible reaction for which the equation of the wave has the symmetrical form shown by equation 15, and it has received ample experimental verification (5, 7, 9, 12, 21).

B. Determination of the coordination number

From equation 16 the half-wave potential of a complex metal ion should shift with changing activity of the complex-forming substance according to

$$\frac{\Delta E_{1/2}}{\Delta \log C_X f_X} = -p \frac{0.0591}{n} \quad (17)$$

This relation is important because it enables us to determine the coordination number p of the complex metal ion, and thus its formula. For this purpose it is usually sufficiently accurate to employ the concentration of X^{-b} in place of its activity.

C. Relation between the half-wave potential and the dissociation constant

From equation 16 we see that the half-wave potential depends on the logarithm of the dissociation constant of the complex metal ion, and it is more negative the smaller the value of K_o , i.e., the more stable the complex ion. Although K_o can be evaluated from the observed value of the half-wave potential itself, it is usually more accurate to determine it from the difference between the $E_{1/2}$ value of the complex metal ion and that of the

corresponding simple metal ion. Lingane (12) and Stackelberg (18) have shown that the half-wave potential of a simple metal ion is expressible by

$$(E_{1/2})_s = \epsilon - \frac{0.0591}{n} \log \frac{f_s k_s}{f_s k_s} \quad (18)$$

where k_s is proportional to the square root of the diffusion coefficient of the simple metal ions, and is defined by equation 10 when $D_{MX}^{1/2}$ is replaced by $D_s^{1/2}$. Hence from equations 16 and 18 it follows that the shift of the half-wave potential by complex formation should obey the relation

$$(E_{1/2})_c - (E_{1/2})_s = \frac{0.0591}{n} \log \frac{K_o f_o k_s}{f_s k_o} - p \frac{0.0591}{n} \log C_X f_X \quad (19)$$

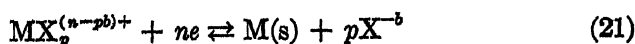
in which the subscripts "c" and "s" refer to the complex and simple metal ions, respectively. In this equation $(E_{1/2})_c$ and $(E_{1/2})_s$ are to be given their proper signs with respect to the particular reference electrode against which they are measured.

Since the best precision with which $E_{1/2}$ values can be measured is about ± 3 millivolts, the retention of activity coefficient terms in the foregoing equations is usually of minor importance. Furthermore, the ratio k_s/k_o , which is equal to $(D_s/D_o)^{1/2}$, is usually so close to 1 that it also may be neglected. Hence for *approximate* purposes equation 19 can be simplified to

$$(E_{1/2})_c - (E_{1/2})_s \cong \frac{0.0591}{n} \log K_o - p \frac{0.0591}{n} \log C_X \quad (20)$$

When a more exact result is desired, the ratio k_s/k_o can be determined experimentally from the ratio of the observed diffusion currents of the simple and complex metal ions at the same concentration and with all other conditions constant.

Instead of employing K_o explicitly, the quantity $\frac{0.0591}{n} \log K_o$ may be replaced by $E_o^0 - E_s^0$, where E_o^0 and E_s^0 are, respectively, the ordinary standard potentials of the reactions



and



Equation 20 then becomes

$$(E_{1/2})_c - (E_{1/2})_s = E_o^0 - E_s^0 - p \frac{0.0591}{n} \log C_X \quad (23)$$

D. Reduction of the bismuthite ion

The reduction of the bismuthite ion in strongly alkaline medium furnishes a good example of the practical application of the foregoing relations. A typical polarogram of $5 \times 10^{-4} M$ lead nitrate in $1.09 N$ sodium hydroxide and $0.8 N$ potassium nitrate is shown in figure 1. To test the reversibility of the reduction, values of $i/(i_d - i)$ were carefully measured from this polarogram and the logarithms of these values are shown plotted against

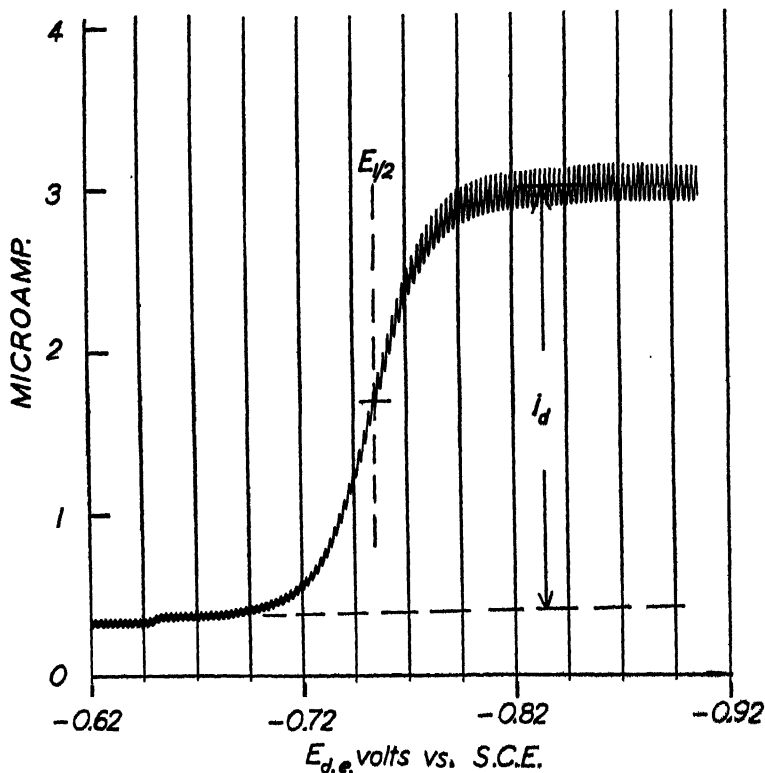


FIG. 1. Polarogram of $5 \times 10^{-4} M$ lead nitrate in $1.09 N$ sodium hydroxide at 25°C .

$E_{d,e}$ in figure 2. As predicted by equation 15, the points of the log plot form a good straight line, whose slope of 0.029 volt is an excellent agreement with the theoretical value, 0.0296 volt, for $n = 2$. It is evident that the reduction of lead from strongly alkaline medium proceeds reversibly at the dropping electrode.

The fact that the lead exists as the HPbO_2^- ion in strongly alkaline solutions is demonstrated by the data in table 1 for the half-wave potential as a function of the hydroxyl-ion concentration. Polarograms were recorded in the usual way with a polarograph of the Heyrovsky-Shikata type (4, 9).

A relatively small concentration of lead was employed ($ca. 5 \times 10^{-4} M$), so that the currents at the midpoints of the waves would be small (about 2 microamperes) and thus keep the iR drop in the cell negligibly small.

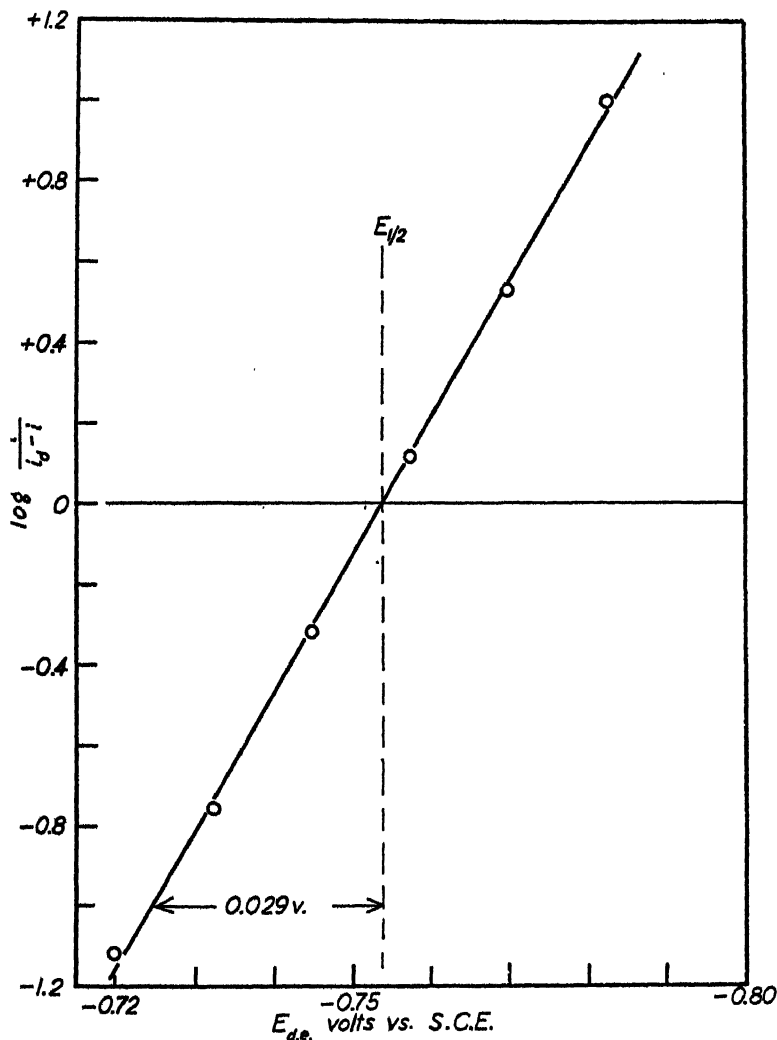


FIG. 2. Test of the equation of the wave of the bismuthite ion. Experimental points from figure 1.

In order to attain maximum precision, the total potential drop across the bridge of the polarograph was adjusted so that each revolution of the drum corresponded to only 0.025 volt of applied E.M.F. A quiet pool of mercury in the cell served as anode, and the cell was provided with a salt bridge in

order that the anode potential could be measured against an external saturated calomel electrode at any time during the experiments (9, 13). The $E_{1/2}$ values were obtained by algebraic subtraction of the anode potential from the half-wave values of the total applied E.M.F. (9). With this technique the $E_{1/2}$ values were reproducible to about ± 3 millivolts. No correction was made for the liquid-junction potential between the cell solution and the saturated potassium chloride solution in the calomel electrode, because experience has shown that this correction is of minor importance for the purpose of determining the value of p . Experiments 1 to 8 in table 1 were run with 0.6 to 1 N potassium nitrate as additional

TABLE 1

Half-wave potential of the biplumbite ion as a function of the hydroxyl-ion concentration
Half-wave potentials with respect to the saturated calomel electrode at 25°C.

NO.	EXCESS NaOH	LOG C_{OH^-}	$E_{1/2}$
In 0.6 to 1 N KNO_3			
	N		volts
1	0.011	-1.95	-0.603
2	0.038	-1.42	-0.649
3	0.060	-1.21	-0.666
4	0.099	-1.00	-0.681
5	0.201	-0.70	-0.708
6	0.448	-0.35	-0.734
7	0.702	-0.15	-0.747
8	1.09	+0.04	-0.755
Without additional supporting electrolyte			
9	0.050	-1.30	-0.658
10	0.124	-0.91	-0.690
11	0.263	-0.58	-0.714
12	0.576	-0.24	-0.736

supporting electrolyte, but in experiments 9 to 12 no additional supporting electrolyte was present. Identical results were obtained in both series.

The data in table 1 are represented graphically in figure 3, and it is seen that the observed $E_{1/2}$ values are a linear function of $\log C_{OH^-}$, as predicted by equations 16 and 17. The observed slope of the $E_{1/2}$ vs. $\log C_{OH^-}$ line is -0.083 volt, in satisfactory agreement with the theoretical value, which is $-\frac{3}{2} \times 0.0591$, or -0.089 volt, for $p = 3$ and $n = 2$. The small deviation of the experimental points from the theoretical slope for $p = 3$ can be attributed to the simplifying assumption that the concentration of the hydroxyl ion was equal to its activity. These data demonstrate that correction for activity coefficients is not necessary for the particular purpose of determining p . It is evident that the ionic state of the lead at hydroxyl-

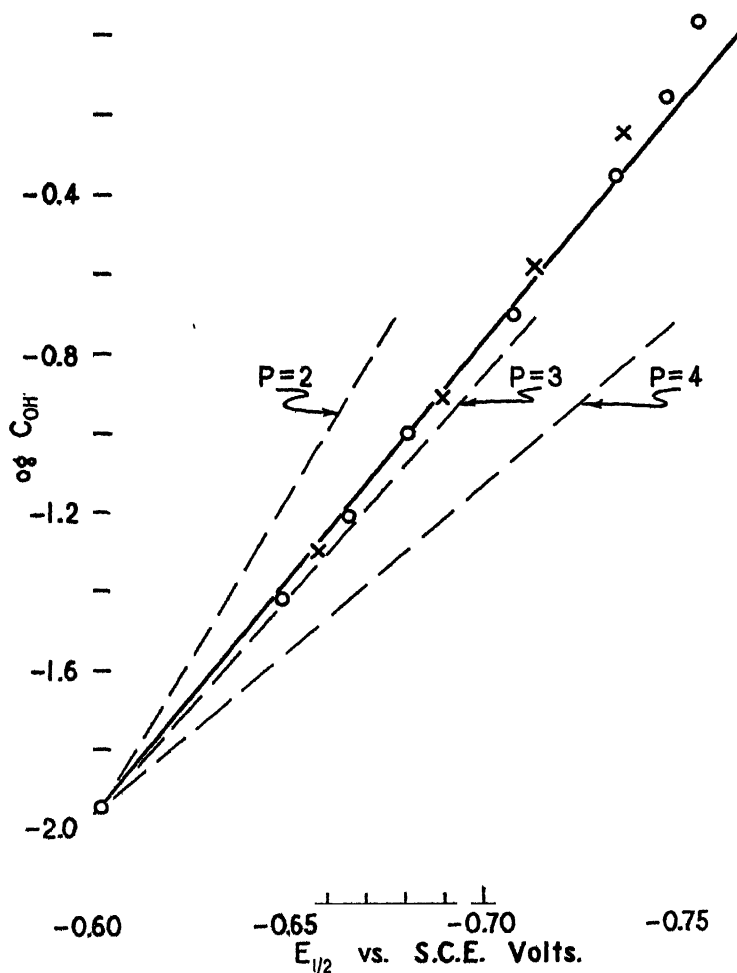
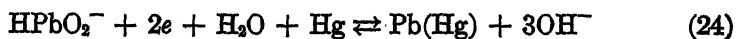


FIG. 3. Half-wave potential of lead in alkaline medium as a function of the hydroxyl-ion concentration. Data from table 1.

ion concentrations from 0.01 to 1 *N* corresponds to the biplumbite ion $HPbO_2^-$, which is reduced reversibly at the dropping electrode according to²



² According to M. Randall and H. M. Spencer (J. Am. Chem. Soc. 50, 1572 (1928)), the ionic state of lead in sodium hydroxide solutions is $HPbO_2^-$, rather than $Pb(OH)_3^-$.

The half-wave potential of Pb^{++} in 0.1 *N* potassium nitrate is -0.388 volt against the saturated calomel electrode (12), and from table 1 the half-wave potential of HPbO_2^- in 0.1 *N* sodium hydroxide (either with or without potassium nitrate present) is -0.681 volt against the saturated calomel electrode. Thus the observed difference in the half-wave potentials under these conditions is -0.29 volt. In order to calculate the theoretical difference between the half-wave potentials of HPbO_2^- and Pb^{++} we shall employ the following data given by Latimer (11):



From these data and equation 23 we have

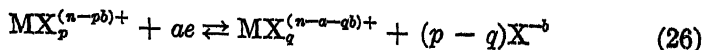
$$(E_{1/2})_{\text{HPbO}_2^-} - (E_{1/2})_{\text{Pb}^{++}} = -0.41 - \frac{3}{2} \times 0.0591 \log C_{\text{OH}^-} \quad (25)$$

This equation predicts that the difference between the half-wave potentials should be -0.32 volt when $C_{\text{OH}^-} = 0.1 M$. The agreement of the observed value (-0.29 volt) with this theoretical value is quite satisfactory. The fact that the observed difference of the half-wave potentials is slightly smaller than the theoretical difference appears to be due chiefly to the fact that the measured half-wave potential of HPbO_2^- includes an appreciable liquid-junction potential between the strongly alkaline cell solution and the saturated potassium chloride solution in the calomel reference electrode. Although the exact value of this liquid-junction potential is unknown, its *direction* is such that it decreases the measured half-wave potential of HPbO_2^- .

The ratio k_s/k_o , determined experimentally from the diffusion currents of equal concentrations of Pb^{++} and HPbO_2^- , respectively, was found to be 1.05, and hence so close to 1 that it was neglected in the foregoing calculations.

III. REDUCTION OR OXIDATION FROM ONE IONIC OXIDATION STATE TO ANOTHER

Various cases are known in which metal ion complexes are reduced to a lower oxidation state, or oxidized to a higher oxidation state, at the dropping electrode. We shall consider a general case in which the net electrode reaction may be represented by



The various kinds of waves that result from this type of reaction are represented schematically in figure 4. In this figure we employ the usual con-

vention of giving a positive sign to a cathodic current (electroreduction) and a negative sign to an anodic current (electrooxidation).

Curve 1 represents the type of wave obtained with a solution that originally contains only the oxidized form, and it is entirely cathodic. Curve 3 represents the other extreme case, in which only the reduced form is originally present in the solution, and it is entirely anodic. The type of

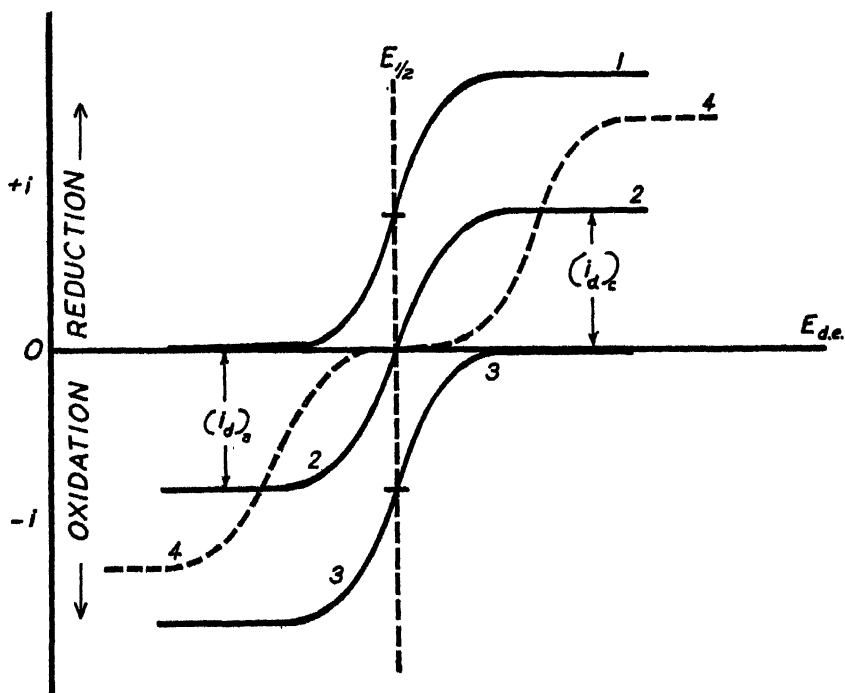


FIG. 4. Schematic representation of polarographic waves due to the reduction or oxidation of metal ions, or other substances, from one oxidation state to another. Curve 1, cathodic wave of oxidized form. Curve 2, composite cathodic-anodic wave of a mixture of the oxidized and reduced forms. Curve 3, anodic wave of the reduced form. Curve 4, composite cathodic-anodic wave of a mixture of the reduced and oxidized forms when the electrode reaction is irreversible.

composite cathodic-anodic curve obtained for a reversible reaction, when the solution contains both the oxidized and reduced forms at about equal concentrations, is shown by curve 2. The cathodic part of the wave above the zero line corresponds to the reduction of the oxidized form (equation 26 from left to right), while the lower anodic part of the wave is due to the oxidation of the reduced form. It should be emphasized that curves 1, 2, and 3 correspond to a reversible reaction. *When this condition is fulfilled,*

the cathodic, anodic, and composite cathodic-anodic waves will have identical half-wave potentials. In such cases the dropping electrode functions simply as an "indifferent" electrode, and its potential at the point where $i = 0$ coincides with the potential that one would observe with a platinum or other noble-metal indicator electrode by the classical potentiometric technique.

When reaction 26 is irreversible at the dropping electrode, the composite cathodic-anodic wave will have the form shown by curve 4. In this case more or less overvoltage is involved in either the cathodic or anodic reactions, or both, and consequently the two half-wave potentials will not be equal.

A. Equation of the waves

A general equation of these waves will be derived on the basis of the following assumptions: (1) that the electrode reaction is reversible; (2) that sufficient supporting electrolyte is present so that the current is entirely diffusion controlled; and (3) that a sufficient excess of the complex-forming substance is present in the solution to insure that its concentration at the electrode surface will remain virtually constant and independent of the current. The potential of the dropping electrode at any point on either a cathodic, an anodic, or a composite wave will then be expressable (assuming concentrations equal to activities) by

$$E_{d.e.} = E^0 - \frac{0.0591}{a} \log \frac{C_{\text{Red.}}^0}{C_{\text{Ox.}}^0} - \left(\frac{p-q}{a} \right) 0.0591 \log C_X \quad (27)$$

In this equation, E^0 is the ordinary standard potential of reaction 26, $C_{\text{Red.}}^0$ and $C_{\text{Ox.}}^0$ are, respectively, the concentrations at the electrode surface of the complex ions in the lower and higher oxidation states, and C_X is the concentration of X^{-b} .

The cathodic diffusion current is directly proportional to the concentration of the oxidized form in the body of the solution,—

$$(i_d)_c = k_{\text{Ox.}} C_{\text{Ox.}} \quad (28)$$

where $k_{\text{Ox.}}$ is defined by equation 10. Similarly, the anodic diffusion current is directly proportional to the concentration of the reduced form in the body of the solution,—

$$-(i_d)_a = k_{\text{Red.}} C_{\text{Red.}} \quad (29)$$

If we assume that the changes in the concentrations of the reduced and oxidized forms at the electrode surface are directly proportional to the current, then at each value of i we have

$$C_{\text{Red.}}^0 = C_{\text{Red.}} + \frac{i}{k_{\text{Red.}}} = \frac{-(i_d)_a + i}{k_{\text{Red.}}} \quad (30)$$

and

$$C_{\text{Ox.}} = C_{\text{Ox.}} - \frac{i}{k_{\text{Ox.}}} = \frac{(i_d)_c - i}{k_{\text{Ox.}}} \quad (31)$$

In these equations i is to be given its proper sign of (+) for the cathodic part of a wave and (−) for the anodic part.

When the foregoing relations are substituted into equation 27 we obtain the following general equation, which is applicable to either a cathodic, an anodic, or a composite wave,

$$E_{d.e.} = E_{1/2} - \frac{0.0591}{a} \log \frac{i - (i_d)_a}{(i_d)_c - i} \quad (32)$$

and the half-wave potential is given by

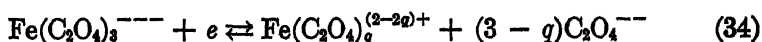
$$E_{1/2} = E^0 - \frac{0.0591}{a} \log \frac{k_{\text{Ox.}}}{k_{\text{Red.}}} - \left(\frac{p - q}{a} \right) 0.0591 \log C_X \quad (33)$$

Since the curve of equation 32 is symmetrical about its midpoint, the half-wave potential should be constant and independent of both the absolute and relative concentrations of the oxidized and reduced forms. The relative values of p and q can be obtained from the shift of $E_{1/2}$ as the concentration of the complex-forming substance is varied. If p happens to be equal to q , then it is evident from equation 33 that the half-wave potential should be constant and independent of the concentration of the complex-forming substance.

It should also be noted that the ratio $k_{\text{Ox.}}/k_{\text{Red.}}$, which is equal to $(D_{\text{Ox.}}/D_{\text{Red.}})^{1/2}$, can be evaluated experimentally from the ratio of the cathodic and anodic diffusion currents obtained with a solution containing equal concentrations of the oxidized and reduced forms. In most cases this ratio is sufficiently close to 1 so that it can be neglected. When this is true, the half-wave potential observed when $C_X = 1 M$ should practically coincide with the ordinary standard oxidation-reduction potential of the system.

B. Ferric and ferrous oxalate complexes

As a practical example of the application of the foregoing relations, we shall consider the reduction and oxidation of the complex ions formed between ferric and ferrous iron and oxalate ion;



The polarographic behavior of this system has been studied by Stackelberg and Freyhold (19). The formula of the ferrous complex is written in the general form because it depends on the concentration of excess oxalate, as will be shown below.

The reversibility of this reaction at the dropping electrode is demonstrated by the polarogram in figure 5 and the log plot in figure 6. This

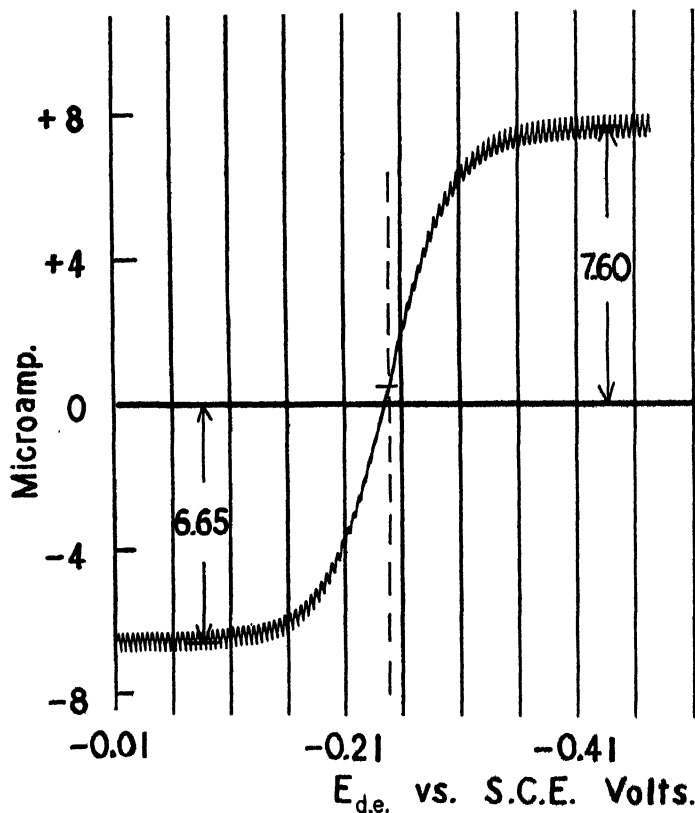


FIG. 5. Composite cathodic-anodic wave of a mixture of approximately 0.001 *M* each of ferric and ferrous iron in 1 *M* potassium oxalate at 25°C.

polarogram was obtained with a mixture of about 0.001 *M* ferric iron and 0.001 *M* ferrous iron in 1 *M* potassium oxalate, and it has the expected shape of a composite cathodic-anodic wave of a reversible reaction. The part of the curve above the galvanometer zero line is due to the reduction of the trioxalato ferric ion, and the lower part to the oxidation of the oxalato ferrous ion. In order to test the reversibility of the reaction, various corresponding values of $E_{d.e.}$ and the quantity $\frac{i - (i_d)_a}{(i_d)_o - i}$ were care-

fully measured from the polarogram in figure 5 and are shown plotted in figure 6. In agreement with equation 32 the experimental points fall on a straight line, whose slope of 0.063 volt is in good agreement with the theoretical value, 0.059 volt, for $\alpha = 1$.

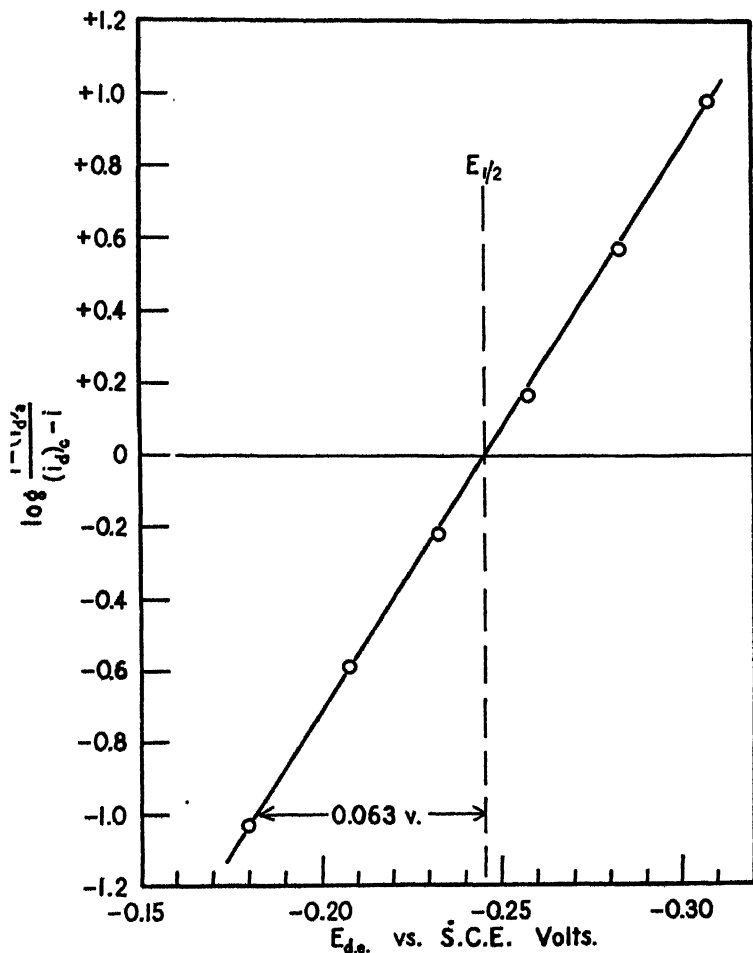


FIG. 6. Test of the reversibility of the ferric-ferrous system in oxalate medium. Experimental points from figure 5.

It will be evident from equation 33, since $k_{Ox.}$ is approximately equal to $k_{Red.}$, that the $E_{1/2}$ of reaction 34 should be practically equal to its ordinary standard potential when $C_{C_2O_4^{2-}} = 1 M$. From figures 5 and 6 the observed half-wave potential in 1 M oxalate is -0.245 volt against the saturated calomel electrode. This agrees reasonably well with the value -0.23 volt (S.C.E.) for the standard potential of this system that Shaper (17) observed

by the classical potentiometric method. The polarographic value actually appears to be more reliable than Shaper's value, since he erroneously assumed that the formula of the ferrous complex was $\text{Fe}(\text{C}_2\text{O}_4)_2^{--}$, independent of the oxalate-ion concentration, and this is correct only when the concentration of excess oxalate is relatively small.

Stackelberg and Freyhold (19) stated that the half-wave potential in the reduction of the trioxalato ferric ion was constant and independent of the concentration of oxalate between 0.01 and 1 *M*. However, Mr. John Scott and the author were unable to confirm this statement. We obtained the data in table 2, which demonstrate that, although $E_{1/2}$ is constant at

TABLE 2

Half-wave potential of trioxalato ferric ion as a function of the oxalate concentration
Each solution contained 0.3 to 0.5 *N* KCl as additional supporting electrolyte.
Concentration of ferric iron was 0.001 to 0.002 *M*. Temperature = 25°C.

$\text{K}_2\text{C}_2\text{O}_4$ IN EXCESS	$\text{LOG } C_{\text{C}_2\text{O}_4^{--}}$	$E_{1/2}$ AG/ CALOMEL ELECTRODE
<i>M</i>		volts
0.0061	-2.21	-0.179
0.0122	-1.91	-0.204
0.0193	-1.71	-0.209
0.0298	-1.53	-0.212
0.053	-1.28	-0.232
0.0747	-1.13	-0.230
0.118	-0.93	-0.236
0.158	-0.80	-0.241
0.171	-0.77	-0.242
0.357	-0.45	-0.240
0.617	-0.21	-0.245
0.817	-0.09	-0.242
1.76	+0.25	-0.242

-0.242 ± 3 volt (S.C.E.) when the concentration of excess oxalate is greater than about 0.15 *M*, with smaller concentrations of oxalate it is shifted to a more positive value as $C_{\text{C}_2\text{O}_4^{--}}$ decreases.

This effect is shown more clearly by the plot of these data in figure 7. The dotted lines are the theoretical slopes predicted by equation 33 for $p - q = 1$ and $p = q$. In view of the large scale of this plot the agreement of the experimental points with the theoretical lines is reasonably satisfactory.

It is evident that $p - q = 1$ when the concentration of excess oxalate is smaller than about 0.15 *M* (with 0.001 to 0.002 *M* ferric iron), and at higher oxalate-ion concentrations q becomes equal to p . Since there is no doubt that the formula of the ferric complex is $\text{Fe}(\text{C}_2\text{O}_4)_3^{--}$, it follows

that the formula of the ferrous complex is $\text{Fe}(\text{C}_2\text{O}_4)_2^{--}$ when $C_{\text{C}_2\text{O}_4^{--}}$ is less than $0.15\text{ }M$, and that the trioxalato ferrous ion, $\text{Fe}(\text{C}_2\text{O}_4)_3^{--}$, is formed only with a larger excess of oxalate ion.

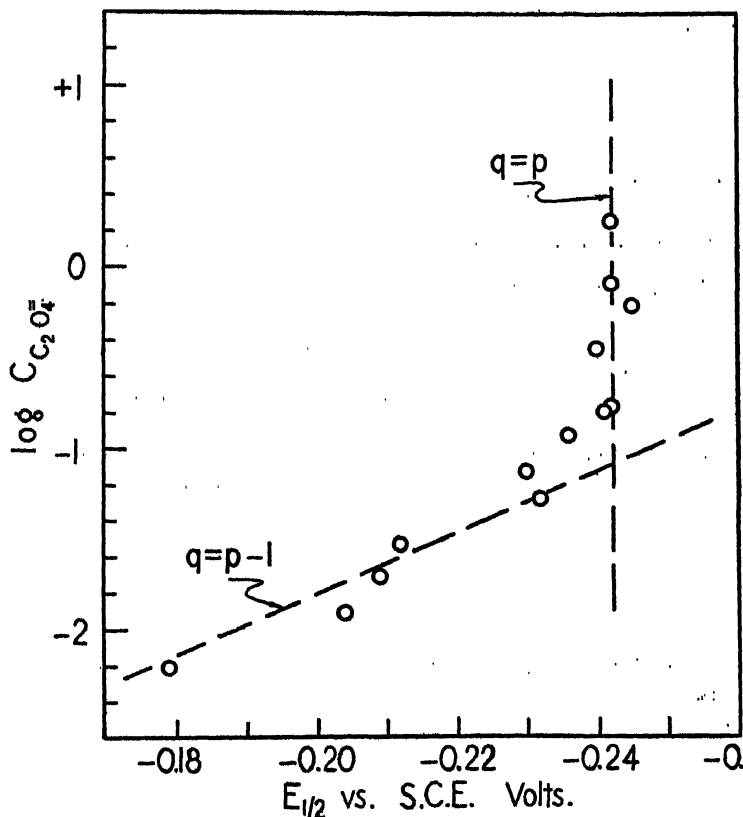


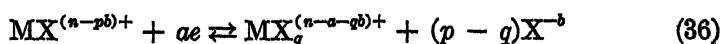
FIG. 7. Half-wave potential of trioxalato ferric ion as a function of the excess oxalate concentration. Data from table 2.

C. Relation between the half-wave potential and the dissociation constants of the oxidized and reduced complex ions

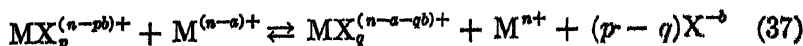
The difference between the half-wave potentials in the partial reduction of a simple metal ion and in the reduction of a complex ion of the same metal can be derived by considering the two corresponding reactions



and



Since the number of electrons involved is the same for both reactions, the difference between their standard potentials is equal to the standard E.M.F. of the reaction



We have

$$E_{37}^0 = E_c^0 - E_s^0 \quad (38)$$

where E_c^0 and E_s^0 are the standard potentials in the reduction of the complex and simple metal ions. In turn, E_{37}^0 is equal to $\frac{RT}{aF_y} \ln K_{37}$, where K_{37} is the equilibrium constant of reaction 37. However, it is evident that

$$K_{37} = \frac{(\text{MX}_q^{(n-a-qb)+})(\text{M}^{n+})(\text{X}^{-b})^{p-q}}{(\text{MX}_p^{(n-pb)+})(\text{M}^{(n-a)+})} = \frac{K_{\text{O.C.}}}{K_{\text{R.C.}}} \quad (39)$$

where $K_{\text{O.C.}}$ and $K_{\text{R.C.}}$ are, respectively, the dissociation constants of the complex ions in the higher and lower oxidation states. It follows, therefore, that

$$E_c^0 - E_s^0 = \frac{RT}{aF_y} \ln K_{37} = \frac{RT}{aF_y} \ln \frac{K_{\text{O.C.}}}{K_{\text{R.C.}}} \quad (40)$$

From reasoning analogous to that in the preceding section, it can be shown that the half-wave potential in the reduction of a *simple* metal ion to a lower oxidation state (equation 35) is related to the ordinary standard potential of the reaction, E_s^0 , by

$$(E_{1/2})_s = E_s^0 - \frac{0.0591}{2a} \log \frac{D_{\text{O.S.}}}{D_{\text{R.S.}}} \quad (41)$$

where $D_{\text{O.S.}}$ and $D_{\text{R.S.}}$ are the diffusion coefficients of the oxidized and reduced forms of the simple metal ion, respectively, and concentrations have been assumed equal to activities. Since $D_{\text{O.S.}}$ and $D_{\text{R.S.}}$ will usually not differ by more than a factor of 1.5, the last term in this equation has only minor significance, and $(E_{1/2})_s$ is practically equal to E_s^0 . For the same reason we may neglect the second term in equation 33, and write, as a good approximation,

$$(E_{1/2})_c = E_c^0 - \left(\frac{p-q}{a} \right) 0.0591 \log C_X \quad (42)$$

From these relations, and equation 40, it follows that

$$(E_{1/2})_o - (E_{1/2})_r \cong E_o^0 - E_r^0 - \left(\frac{p-q}{a}\right) 0.0591 \log C_X \quad (43)$$

or

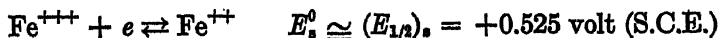
$$(E_{1/2})_o - (E_{1/2})_r \cong \frac{0.0591}{a} \log \frac{K_{O.C.}}{K_{R.C.}} - \left(\frac{p-q}{a}\right) 0.0591 \log C_X \quad (44)$$

The shift of the half-wave potential thus depends on the *ratio* of the dissociation constants of the oxidized and reduced complex ions, and also, of course, on the concentration of the complex-forming substance. If, as is usually the case, the oxidized complex ion is considerably more stable than the corresponding reduced form, then $K_{O.C.}$ is smaller than $K_{R.C.}$, and $(E_{1/2})_o$ is more negative than $(E_{1/2})_r$, when $C_X = 1 M$. If in a particular case $K_{O.C.}$ and $K_{R.C.}$ should happen to be equal, then $(E_{1/2})_o$ would be more *positive* than $(E_{1/2})_r$ when C_X was less than 1 molar; the two half-wave potentials would be equal when $C_X = 1 M$; and $(E_{1/2})_o$ would become more *negative* than $(E_{1/2})_r$ only when C_X was greater than 1 M . In general, when

$$(C_X)^{p-q} \begin{matrix} \geq \\ < \end{matrix} \frac{K_{O.C.}}{K_{R.C.}}$$

then $(E_{1/2})_o - (E_{1/2})_r \begin{matrix} \geq \\ < \end{matrix} 0$.

These relations, combined with the data in table 2, may be used to evaluate the dissociation constants of the trioxalato ferric ion and the dioxalato ferrous ion. For the reduction of the simple ferric ion we have (11)



This value is so much more positive than the potential at which anodic dissolution of mercury takes place from the dropping electrode that it is impossible to measure the half-wave potential of ferric ion directly (10). For the reduction of the trioxalato ferric ion $(E_{1/2})_o = -0.242$ volt (S.C.E.), and $p = q$ when the oxalate concentration is greater than about 0.15 M . Hence from equation 44 we have

$$0.0591 \log \frac{K_{Fe(C_2O_4)_3^-}}{K_{Fe(C_2O_4)_2^-}} = -0.242 - 0.525 = -0.767 \text{ volt}$$

and therefore

$$\frac{K_{Fe(C_2O_4)_3^-}}{K_{Fe(C_2O_4)_2^-}} = 1 \times 10^{-13} \quad (45)$$

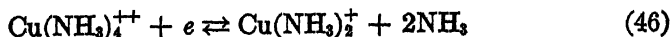
From data obtained by Shaper (17) on the solubility of ferrous oxalate in 0.25 to 1.4 *M* potassium oxalate solutions, the dissociation constant of the trioxalato ferrous ion is found to be 6.1×10^{-7} . Combining this value with equation 45, the dissociation constant of the trioxalato ferric ion is calculated to be 6×10^{-20} .

An approximate value for the dissociation constant of the dioxalato ferrous ion can be obtained from the $E_{1/2}$ data in table 2 at oxalate-ion concentrations less than 0.15 *M*. For example, in 0.0193 *M* oxalate $(E_{1/2})_0$ is equal to -0.209 volt, and $p - q = 1$. Hence from equation 44 we obtain

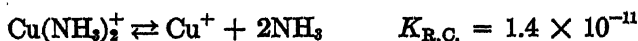
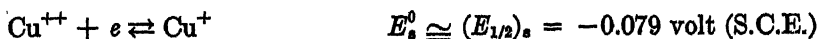
$$\frac{K_{\text{Fe}(\text{C}_2\text{O}_4)_2^-}}{K_{\text{Fe}(\text{C}_2\text{O}_4)_3^-}} = 8 \times 10^{-15}$$

and employing the above value of 6×10^{-20} for $K_{\text{Fe}(\text{C}_2\text{O}_4)_3^-}$, the dissociation constant of the dioxalato ferrous ion comes out to be about 8×10^{-6} .

The $\text{Cu}(\text{NH}_3)_4^{++} \rightarrow \text{Cu}(\text{NH}_3)_2^+$ system furnishes another test of equation 44. The half-wave potential of the reaction



is -0.214 volt (S.C.E.) in a supporting electrolyte composed of 0.2 *N* ammonium perchlorate and 1 *N* ammonia (see figure 9). It is impossible to measure experimentally the half-wave potential of the reaction $\text{Cu}^{++} \rightarrow \text{Cu}^+$, because its value is more negative than that of the reaction $\text{Cu}^{++} \rightarrow \text{Cu}(\text{Hg})$ (compare equations 62 and 63), and hence in non-complex-forming supporting electrolytes simple cupric ions produce only a single wave corresponding to the latter reaction. However, if the reduction of the simple Cu^{++} ion is reversible, the hypothetical $E_{1/2}$ value of the reaction $\text{Cu}^{++} \rightarrow \text{Cu}^+$ will be practically equal to its standard potential. From data compiled by Latimer (11) we have



When these data are substituted into equation 44 we obtain

$$(E_{1/2})_0 = -0.079 + 0.0591 \log \frac{4.6 \times 10^{-14}}{1.4 \times 10^{-11}} = -0.226 \text{ volt (S.C.E.)}$$

as the theoretical half-wave potential of reaction 46 when $C_{\text{NH}_3} = 1$ molar. This value agrees sufficiently well with the observed value, -0.214 volt, to demonstrate the general validity of equation 44. It should also be

noted that in 1 *M* ammonia only about three-fourths of the cupric copper is present as the tetrammino cupric ion, and the remainder is present as the pentammino cupric ion $\text{Cu}(\text{NH}_3)_5^{++}$ (see table 3), which was neglected in the foregoing calculations.

TABLE 3

Dissociation constants of the various ammonia complexes of cuprous and cupric ions, and their relative proportions as a function of the excess ammonia concentration (according to J. Bjerrum)

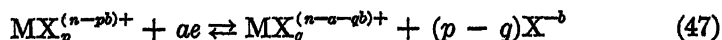
The figures given are the relative proportions of the various ionic species calculated from the indicated dissociation constants. A dash signifies that the proportion of the ion is less than 0.01

NH_3 MOLARITY	CUPROUS COMPLEXES		
	$\text{Cu}(\text{NH}_3)^+$	$\text{Cu}(\text{NH}_3)_2^+$	
	$K_c = 1.2 \times 10^{-8}$	$K_c = 1.35 \times 10^{-11}$	
1	—	1.00	
0.01	—	1.00	
0.001	0.01	0.99	
10^{-4}	0.11	0.89	

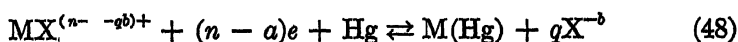
NH_3 MOLARITY	CUPRIC COMPLEXES					
	Cu^{++}	$\text{Cu}(\text{NH}_3)^{++}$	$\text{Cu}(\text{NH}_3)_2^{++}$	$\text{Cu}(\text{NH}_3)_3^{++}$	$\text{Cu}(\text{NH}_3)_4^{++}$	$\text{Cu}(\text{NH}_3)_5^{++}$
		$K_c = 4.9 \times 10^{-8}$	$K_c = 1.05 \times 10^{-8}$	$K_c = 9.5 \times 10^{-13}$	$K_c = 4.7 \times 10^{-14}$	$K_c = 1.36 \times 10^{-13}$
1	—	—	—	—	0.74	0.26
0.01	—	—	0.03	0.32	0.65	—
0.001	—	0.08	0.40	0.44	0.08	—
10^{-4}	0.24	0.50	0.23	0.03	—	—
10^{-5}	0.82	0.17	0.01	—	—	—

IV. STEPWISE REDUCTION OF COMPLEX METAL IONS

When the free energies of the various possible oxidation states of a complex metal ion are sufficiently different, reduction from a higher to a lower oxidation state can proceed in stages at the dropping electrode to produce a polarogram consisting of two or more separate waves. We shall consider a general case in which the polarogram consists of two waves, as shown in figure 8, which correspond to the two consecutive reactions



and



If these reactions are reversible at the dropping electrode, the potential of the latter at points on the first wave will be governed by reaction 47, and it will obey the relation

$$E_{d.s.} = E_1^0 - \frac{RT}{n} \ln \frac{C_{\text{Red.}}^0}{C_X^{(p-a)}} \quad (49)$$

where E_1^0 is the standard potential of reaction 47. It will be evident that the first wave corresponds in every respect to the case discussed in the

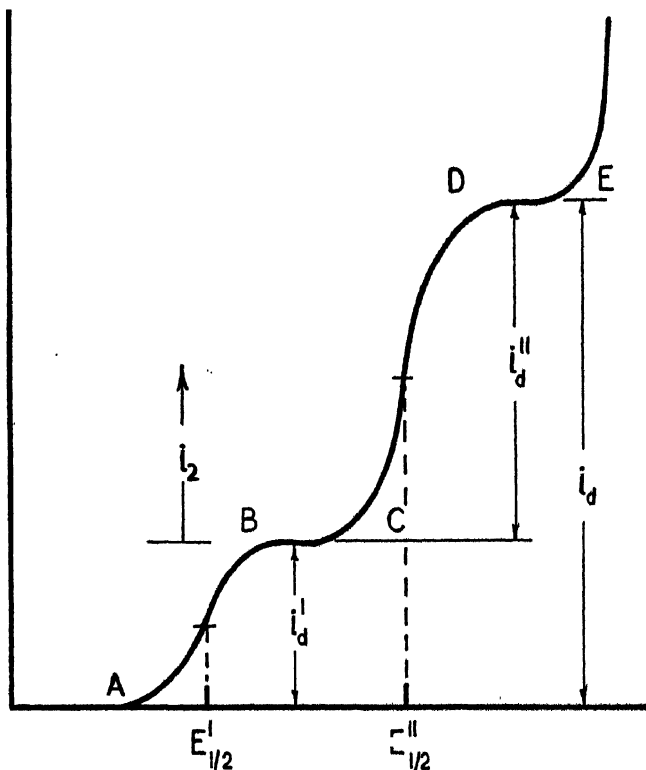


FIG. 8. Schematic polarogram of a stepwise reduction

preceding section. Hence the equation of the first wave will be equation 32 (with $(i_d)_0$ equal to zero), and the half-wave potential of the first wave ($E_{1/2}$ in figure 8) will be given by equation 33.

At points on the second wave the potential of the dropping electrode is governed by reaction 48, and is given by

$$E_{d.s.} = E_2^0 - \frac{RT}{(n-a)F} \ln \frac{C_X^0 C_X^I}{C_{\text{Red.}}^0} \quad (50)$$

where E_2^0 is the standard potential of reaction 48.

Although the potential of the dropping electrode is governed by different reactions on the first and second waves, the *current* at any point on *either* wave is controlled by the rate of diffusion of the oxidized complex ions. If we neglect the slight effect on the diffusion current of the decrease in drop time with increasing negative potential (8, 9, 13), then it follows from the Ilkovič equation (equation 19) that the following relations will hold for the first and second diffusion currents *taken separately*,

$$i'_d = k'aD_{Ox}^{1/2}C_{Ox} = k_1C_{Ox} \quad (51)$$

and

$$i''_d = k'(n - a)D_{Ox}^{1/2}C_{Ox} = k_2C_{Ox} \quad (52)$$

where in both cases D_{Ox} is the diffusion coefficient of the oxidized form, and C_{Ox} is its concentration in the body of the solution. The ratio of the two separate diffusion currents is equal to $a(n - a)$. The *total* diffusion current at *DE* in figure 8 is given by

$$i_d = i'_d + i''_d = k'nD_{Ox}^{1/2}C_{Ox} = kC_{Ox} \quad (53)$$

At points on the first wave (*A* to *B*, figure 8) the concentration of the reduced complex ions at the surface of the dropping electrode is given by

$$(C_{Red}^0)_{A \rightarrow B} = \frac{i}{k_{Red}} \quad (54)$$

When the first diffusion current is reached (*B* to *C* in figure 8) C_{Red}^0 attains a maximal value, and from equations 51 and 54 this maximal value is expressible by

$$(C_{Red}^0)_{B \rightarrow C} = \frac{i'_d}{k_{Red}} = \frac{k_1C_{Ox}}{k_{Red}} = \left(\frac{D_{Ox}}{D_{Red}}\right)^{1/2} C_{Ox} \quad (55)$$

If the diffusion coefficients of the oxidized and reduced forms happen to be equal, then the maximal concentration of the reduced form present at the electrode surface when the first diffusion current is reached will be equal to the concentration of the oxidized form in the body of the solution.

As the potential is increased beyond point *C* the value of C_{Red}^0 decreases again, and it finally becomes practically equal to zero when the second diffusion current is reached at *D*. On the other hand, the concentration of the oxidized form at the electrode surface will have decreased to practically zero when point *B* is reached, and it will remain negligibly small at all values of the potential beyond *B*. If we assume that C_{Red}^0 decreases in direct proportion to the increase in current (in excess of i'_d) at points on the second wave, we have

$$(C_{Red}^0)_{C \rightarrow D} = \frac{i'_d}{k_{Red}} - bi_2 \quad (56)$$

where i_2 is the total current minus i'_d (see figure 8). Since $C_{\text{Red.}}^0$ becomes practically zero at point D , it is evident that the constant b is given by

$$b = \frac{i'_d}{k_{\text{Red.}} i_d''} = \frac{a}{k_{\text{Red.}}(n - a)} \quad (57)$$

and hence equation 56 becomes

$$(C_{\text{Red.}}^0)_{c \rightarrow D} = \frac{i'_d}{k_{\text{Red.}} i_d''} (i_d'' - i_2) = \frac{a}{k_{\text{Red.}}(n - a)} (i_d'' - i_2) \quad (58)$$

When these relations are substituted into equation 50, we obtain for the equation of the second wave, when an excess of X^{-b} is present,

$$E_{d.e.} = E_{1/2}'' - \frac{0.0591}{(n - a)} \log \frac{i_2}{i_d'' - i_2} \quad (59)$$

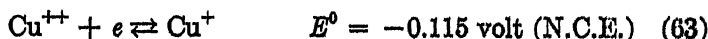
where $E_{1/2}''$ is given by

$$E_{1/2}'' = E_2^0 - \frac{0.0591}{(n - a)} \log \frac{k_{\text{Red.}}(n - a)}{k_a a} - q \frac{0.0591}{(n - a)} \log C_X \quad (60)$$

A. Copper-ammonia complexes

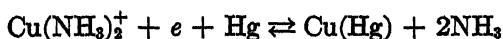
The double waves obtained in the reduction of various cupric complexes serve as good examples to test the foregoing relations, particularly the double wave in ammoniacal medium, which has been studied in detail by Stackelberg and Freyhold (19).

In the absence of complex-forming substances, simple cupric ions (actually tetraquo cupric ions, $\text{Cu}(\text{H}_2\text{O})_4^{++}$) are reduced directly to the metallic state at the dropping electrode and the polarogram shows only a single wave. The reason for this is evident from the standard potentials of the following reactions,



The potentials of reactions 61 and 62 were calculated from the standard potentials of the corresponding reactions involving the solid metal (11) and the known solubility of copper in mercury, by means of relations that have been discussed elsewhere (12, 18). Since the potential of the $\text{Cu}^+ \rightarrow \text{Cu}(\text{Hg})$ system is more positive than that of the $\text{Cu}^{++} \rightarrow \text{Cu}(\text{Hg})$ system, cuprous ions are incapable of stable existence at the potential at which cupric ions are reduced, and hence the polarogram of a solution of simple cupric ions shows only the single wave corresponding to $\text{Cu}^{++} \rightarrow \text{Cu}(\text{Hg})$.

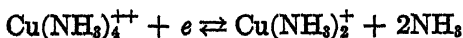
However, if some substance which forms stable complex ions with cupric and cuprous ions, or with cuprous ions alone, is added to a cupric salt solution, then the standard potential and the half-wave potential of the $\text{Cu}^+ \rightarrow \text{Cu}(\text{Hg})$ system will usually be shifted in the negative direction to a greater extent than that of the $\text{Cu}^{++} \rightarrow \text{Cu}^+$ system, and the polarogram shows a double wave. This is due to the fact that the shift of the $\text{Cu}^{++} \rightarrow \text{Cu}^+$ half-wave potential is proportional to $\log \frac{K_{\text{O.C.}}}{K_{\text{R.C.}}}$ (see equation 44), whereas the shift of the half-wave potential of the $\text{Cu}^+ \rightarrow \text{Cu}(\text{Hg})$ system is proportional to $\log K_{\text{R.C.}}$ alone (see equation 20), which ordinarily is a much larger negative quantity than $\log \frac{K_{\text{O.C.}}}{K_{\text{R.C.}}}$. The effect of this unequal shift is evident by comparing the foregoing standard potentials of the simple cupric and cuprous ions with the values for the corresponding ammonia complexes, which are as follows (11, 19):



$$E^0 = -0.522 \text{ volt (N.C.E.)} \quad (64)$$



$$E^0 = -0.397 \text{ volt (N.C.E.)} \quad (65)$$



$$E^0 = -0.262 \text{ volt (N.C.E.)} \quad (66)$$

The order of these potentials is just the reverse of those for the corresponding simple ions.

In 1 molar ammonia solution the potential required to reduce the $\text{Cu}(\text{NH}_3)_2^+$ ion is so much more negative than that at which the $\text{Cu}(\text{NH}_3)_4^{++}$ ion is reduced to $\text{Cu}(\text{NH}_3)_2^+$ that the polarogram consists of two separate waves of equal height, as shown in figure 9. The first wave corresponds to $\text{Cu}(\text{NH}_3)_4^{++} \rightarrow \text{Cu}(\text{NH}_3)_2^+$, and the second to $\text{Cu}(\text{NH}_3)_2^+ \rightarrow \text{Cu}(\text{Hg})$. Similar double waves are obtained with the thiocyanate (14), pyridine (14), and chloride complexes (19) of copper.

The reversibility of both stages of the reduction of the $\text{Cu}(\text{NH}_3)_4^{++}$ ion is easily demonstrable by comparing the difference in potential between two given points on each of the two waves in figure 9 with the theoretical values predicted by the equations of the waves. The values of $E_{d.s.}$ at $i = (1/4)i_d$ and $i = (3/4)i_d$ are convenient reference points. Whenever the equation of the wave of any type of reaction has the familiar symmetrical form

$$E_{d.s.} = E_{1/2} - \frac{0.0591}{n} \log \frac{i}{i_d - i} \quad (67)$$

then

$$E_{3/4} - E_{1/4} = -\frac{0.0591}{n} \log \frac{3}{1/3} \quad \frac{0.058}{n} \quad (68)$$

This relation was originally suggested by Tomeš (21) as a criterion of reversibility.

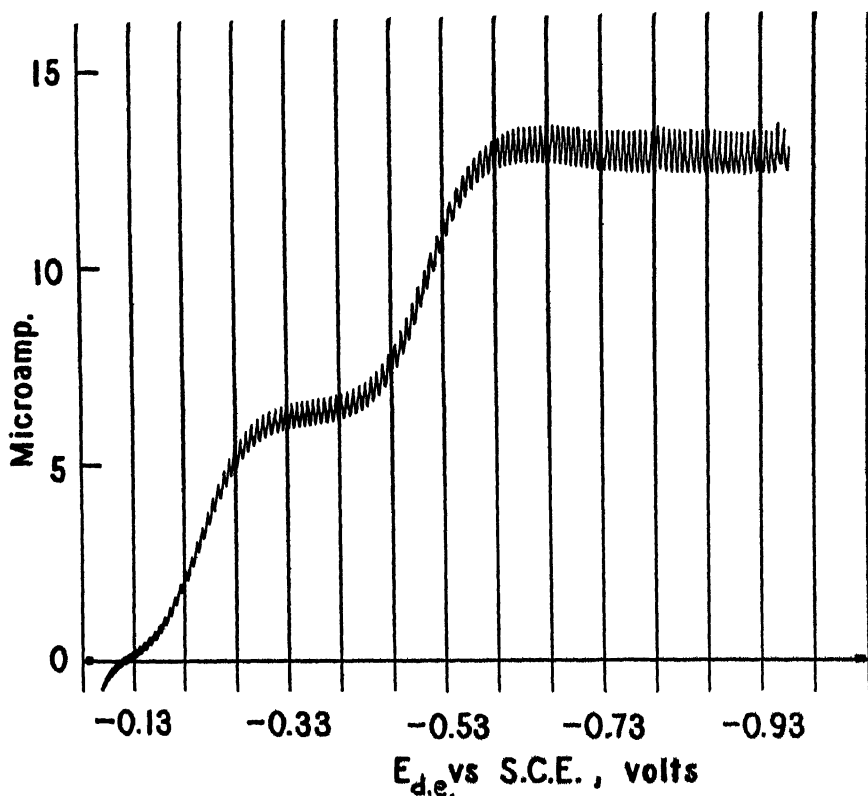


FIG. 9. Polarogram of a solution of $0.002 M \text{ Cu}(\text{NH}_3)_4^{++}$ in $0.2 N$ ammonium perchlorate and $1 N$ ammonia, plus 0.01 per cent gelatin as a maximum suppressor.

In the reduction of the $\text{Cu}(\text{NH}_3)_4^{++}$ ion each wave is due to a one electron change, and hence for each wave $E_{3/4} - E_{1/4}$ should be equal to -0.058 volt. From the polarogram in figure 9 the observed values of $E_{3/4} - E_{1/4}$ are -0.064 volt for the first wave and -0.058 volt for the second, in good agreement with the theoretical value.

Stackelberg and Freyhold (19) investigated the influence of the ammonia concentration on the half-wave potentials of the ammonia-cupric complex. Their experimental results are shown in figure 10, in which the circles are

experimental points. The curves in this graph are the theoretical curves calculated by Stackelberg and Freyhold. The agreement of the experimental points with the theoretical curves is quite satisfactory.

In the calculation of the theoretical curves in figure 10, Stackelberg and Freyhold took account of the fact that cupric ion forms a series of different ammonia complexes from $\text{Cu}(\text{NH}_3)^{++}$ to $\text{Cu}(\text{NH}_3)_6^{++}$, and that cuprous

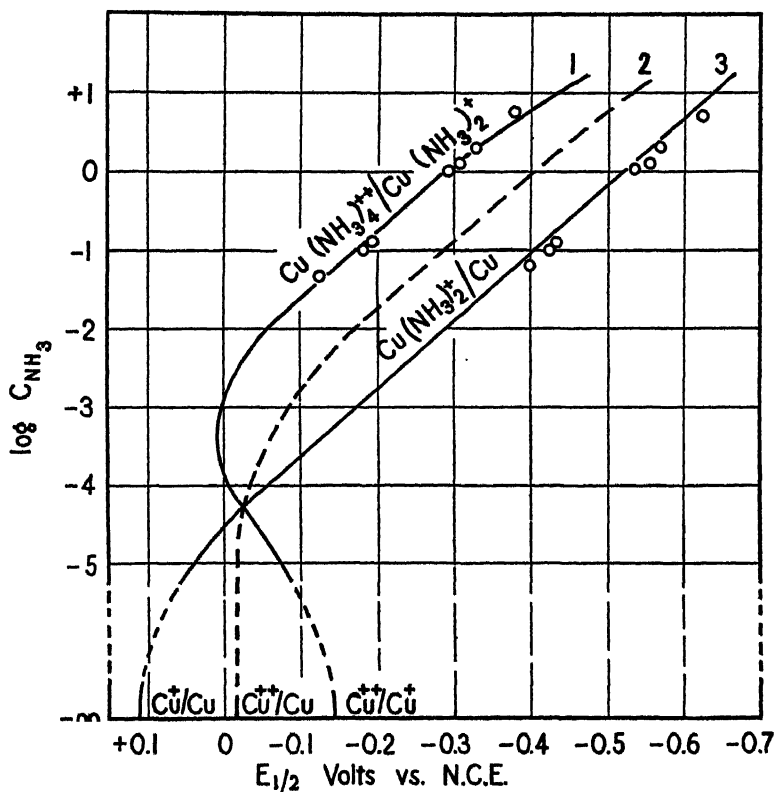


FIG. 10. Half-wave potentials of tetrammino cupric ion and diammino cuprous ion as a function of the ammonia concentration. The lines are theoretical curves, and the circles are experimental points (according to Stackelberg and Freyhold).

ion forms the two complexes $\text{Cu}(\text{NH}_3)^+$ and $\text{Cu}(\text{NH}_3)_2^+$, depending on the concentration of excess ammonia. From the dissociation constants of these various complexes, as determined by J. Bjerrum (1), their relative proportions in different concentrations of excess ammonia can be calculated. This is shown in table 3, which is an expansion of a similar tabulation given by Stackelberg and Freyhold.

It is seen that the predominant cuprous complex is the diammino cuprous

ion, and the proportion of the monammino cuprous ion is negligible for the present purpose. In the case of the cupric complexes, the tetrammino ion $\text{Cu}(\text{NH}_3)_4^{++}$ predominates at concentrations of ammonia between about 0.05 and 1 M . When the concentration of excess ammonia is decreased below 0.05 M the lower order complexes are formed in increasing amount, and at 10^{-4} M ammonia the monammino cupric ion, $\text{Cu}(\text{NH}_3)^{++}$, predominates. These facts are responsible for the *positive* shift of the half-wave potential of the $\text{Cu}^{++} \rightarrow \text{Cu}^+$ system at very small concentrations of ammonia, shown by curve 1 in figure 10.

From equation 44 the shift of the half-wave potential of the $\text{Cu}^{++} \rightarrow \text{Cu}^+$ system by formation of the ammonia complexes should follow the equation

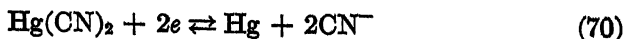
$$(E_{1/2})_0 - (E_{1/2})_s = 0.0591 \log \frac{K_{10}}{K_{\text{ous}}} - (p - q)0.0591 \log C_{\text{NH}_3} \quad (69)$$

where K_{10} and K_{ous} are the dissociation constants of the particular cupric and cuprous complexes that predominate at a given concentration of ammonia. The positive shift of the $E_{1/2}$ of the $\text{Cu}^{++} \rightarrow \text{Cu}^+$ system at very small concentrations of excess ammonia is due to two factors. In the first place, when C_{NH_3} is less than about 5×10^{-4} molar the predominant cupric complexes are the $\text{Cu}(\text{NH}_3)_2^{++}$ and $\text{Cu}(\text{NH}_3)^{++}$ ions, and since both of these complexes have larger dissociation constants than the diammino cuprous ion, the quantity $\log \frac{K_{10}}{K_{\text{ous}}}$ in equation 69 has a positive value when they predominate. Furthermore, at small concentrations of ammonia where the $\text{Cu}(\text{NH}_3)_2^{++}$ and $\text{Cu}(\text{NH}_3)^{++}$ ions predominate, $p - q$ in equation 69 becomes equal to 0 and -1 , respectively. Both of these factors shift $E_{1/2}$ in the positive direction. The inflection point in curve 1 at C_{NH_3} equal to about 5×10^{-4} M is due to the fact that the $\text{Cu}(\text{NH}_3)_3^{++}$ ion predominates, and hence $p = q$ at this concentration of ammonia.

The dotted curve 2 is the hypothetical curve corresponding to direct reduction of the ammonia-cupric complexes to the metallic state (analog). This curve is unattainable experimentally, because no $\text{Cu}(\text{NH}_3)_4^{++}$ ions exist at the surface of the dropping electrode, and the copper is present entirely as the diammino cuprous ion, at potentials more negative than that at which the first diffusion current is reached.

V. ANALYSIS OF THE WAVE OF AN INCOMPLETELY DISSOCIATED METAL SALT: MERCURIC CYANIDE

Tomeš (21) has made a careful polarographic study of the reduction of the weak electrolyte mercuric cyanide, a case which has much in common with the reduction of complex metal ions. The net electrode reaction which controls the current is



Since the activity of the deposited mercury is constant, the potential of the dropping electrode will be given by

$$E_{d.e.} = E^0 - \frac{0.0591}{2} \log \frac{(C_{\text{CN}^-}^0)^2}{C_{\text{Hg}(\text{CN})_2}^0} \quad (71)$$

where E^0 is the standard potential of reaction 70.

Tomeš (21) studied the reduction of mercuric cyanide in buffered solutions *that did not contain excess cyanide*, and under this condition the concentration of CN^- at the electrode surface is a function of the current. Since hydrocyanic acid is very weak ($K_a = 7.2 \times 10^{-10}$), the CN^- ions liberated at the electrode surface are more or less completely hydrolyzed to HCN , depending on the pH of the solution. At any given pH value we have

$$C_{\text{CN}^-}^0 = K_a \frac{C_{\text{HCN}}^0}{C_{\text{H}^+}^0} = 7.2 \times 10^{-10} \frac{C_{\text{HCN}}^0}{C_{\text{H}^+}^0} \quad (72)$$

If we represent the sum of the concentrations of CN^- and HCN at the electrode surface by C^* , then

$$C^* = C_{\text{HCN}}^0 + C_{\text{CN}^-}^0 = \frac{i}{k_1} \quad (73)$$

and, combining this relation with equation 72, we obtain

$$C_{\text{CN}^-}^0 = \frac{K_a i}{(K_a + C_{\text{H}^+}^0) k_1} \quad (74)$$

It will also be evident that

$$C_{\text{Hg}(\text{CN})_2}^0 = \frac{i_d - i}{k_2} \quad (75)$$

When these relations are substituted into equation 71 we obtain, for the equation of the wave when the solution does not contain excess cyanide,

$$E_{d.e.} = \epsilon' - 0.0591 \log \frac{K_a}{K_a + C_{\text{H}^+}^0} - 0.0296 \log \frac{i_d^2}{i_d - i} \quad (76)$$

where the constant ϵ' is equal to $E^0 - 0.0296 \log \frac{k_1}{k_2}$.

Equation 76 predicts that the wave of mercuric cyanide should have the following characteristics: (1) In a well-buffered solution a plot of $E_{d.e.}$ versus $\log \frac{i_d^2}{i_d - i}$, should produce a straight line with a slope of 0.0296 volt at 25°C. (2) Because of the i_d^2 term, the wave is unsymmetrical about its midpoint and the half-wave potential should shift by 0.0296 volt to a more

negative value for a tenfold increase in the concentration of mercuric cyanide. (3) Since K_a is about 10^{-9} , it becomes negligibly small compared to $C_{\text{H}^+}^0$ when the pH is smaller than about 8. Hence, at pH values less than about 8, the half-wave potential should shift by 0.059 volt to a more positive value for each unit decrease in the pH of the buffer, provided that the concentration of mercuric cyanide is kept constant. On the other hand, when the pH is greater than about 10, $C_{\text{H}^+}^0$ becomes negligibly small compared to K_a , and hence, at a constant concentration of mercuric cyanide, the half-wave potential should become constant and independent of the pH when the latter is greater than 10.

The experimental data obtained by Tomeš are in good agreement with these predictions at pH values less than 10. In more alkaline solutions Tomeš observed deviations from the theoretical relations, which he logically ascribed to the formation of complex $\text{Hg}(\text{CN})_4^{--}$ ions at the electrode surface, due to the relatively large proportion of free CN^- ions present at a pH greater than 10. The formation of this complex was neglected in the foregoing derivations, but it will be evident that it could be taken into account from a consideration of the equilibrium $\text{Hg}(\text{CH})_2 + 2\text{CN}^- = \text{Hg}(\text{CN})_4^{--}$.

VI. POLAROGRAPHIC WAVES DUE TO IRREVERSIBLE REDUCTION OF COMPLEX METAL IONS

The foregoing derivations were based on the assumption that the dissociation and reduction of complex metal ions are rapid reversible processes. Although, as already shown, this assumption is valid in many cases, it is not always true; the rate of formation and dissociation of certain complex metal ions is often quite slow. In the reduction or oxidation of such complexes at the dropping electrode, other polarization effects in addition to concentration polarization come into play, and an exact interpretation of the wave becomes very difficult. In such cases the equation of the wave will involve, among other factors, the rate constant of the slow step in the electrode reaction. Since a satisfactory quantitative analysis of such irreversible waves has not yet been obtained, the following remarks will be limited to a brief description of some of the experimental observations that have been reported.

The slow dissociation of certain complex metal ions is strikingly illustrated by the results obtained by Pines (15) in his polarographic study of the cyanide complexes of zinc. Pines found that the polarograms of saturated solutions of zinc cyanide in 0.001 to 0.01 *N* potassium cyanide consist of three distinct waves, with $E_{1/2}$ values of -1.0 volt, -1.2 volts, and -1.4 volts against the saturated calomel electrode. These waves obviously cannot be due to stepwise reduction to intermediate oxidation states since none exist, and Pines concluded that they were due to the reduction of

three different species of zinc-cyanide complexes whose rate of dissociation and interconversion is so slow that each one produces its own characteristic wave. This conclusion is strengthened by further experiments performed by Pines, in which he added increasing amounts of potassium cyanide to a $5 \times 10^{-4} M$ zinc chloride solution (no other supporting electrolyte). When the concentration of cyanide was smaller than $4 \times 10^{-4} N$, the polarogram showed only the first wave at -1.0 volt. When the cyanide-ion concentration was increased above $4 \times 10^{-4} N$, the height of the first wave steadily decreased, and the two other waves at -1.2 and -1.4 volts appeared and gradually increased in height until the cyanide-ion concentration was about $2 \times 10^{-3} N$, at which point the first wave had disappeared. With further increase in the concentration of cyanide, the remaining waves at -1.2 volts and -1.4 volts decreased in height, and the wave at -1.2 volts disappeared when the cyanide concentration was greater than about $0.003 N$. With further increase in the cyanide concentration the last remaining wave at -1.4 volts also disappeared, and no wave at all was observed when the concentration of cyanide was greater than ten times that of the zinc salt. Pines concluded that the first wave at -1.0 volt is due to the reduction of simple Zn^{++} ions, the second at -1.2 volts to the $Zn(CN)_4^{--}$ ion, the third at -1.4 volts to $Zn(CN)_5^{---}$, and with a large excess of cyanide $Zn(CN)_6^{----}$ is apparently formed, the reduction potential of which is more negative than that of potassium ions. The fact that separate waves were observed shows that the rate of conversion of one complex into the other is relatively slow, as otherwise the higher complexes would simply have dissociated into the lower more easily reducible one when its reduction potential was reached, and only a single wave would have been obtained.

It should also be mentioned that Herman (3) has obtained polarographic evidence that the rates of dissociation of gold-cyanide and gold-hydroxide complexes, and the rate of conversion of one complex into the other, are relatively slow. When excess cyanide was added to an alkaline gold solution the characteristic wave of the auric-hydroxide complex persisted, and when excess sodium hydroxide was added to a solution containing the auric-cyanide complex the characteristic wave of the cyanide complex remained unchanged. The latter result is not surprising, since the cyanide complex is the more stable of the two, but the first experiment shows that the rate of conversion of the hydroxide complex into the cyanide complex is very slow.

Some observations of Brdička (2) are also of interest in this connection. In neutral solutions of cobaltous chloride he observed a small "pre-wave" which preceded the main cobaltous wave by about 0.2 volt. The pre-wave disappeared when the solution was acidified, which led Brdička to conclude that it was due to the reduction of the pentaquo-hydroxy cobaltous ion,

$[\text{Co}(\text{H}_2\text{O})_5\text{OH}]^+$, formed by hydrolysis of the hexaquo cobaltous ion. The fact that the pre-wave was obtained indicates that the equilibrium



is established quite slowly.

The reduction of the aquo complex ions of certain heavy metals is often more irreversible than that of the other complexes of these metals. For example, in non-complex-forming supporting electrolytes the half-wave potential of the hexaquo nickel ion is -1.05 volt against the saturated calomel electrode, a value more than 0.5 volt more negative than one would predict from the standard potential of nickel. This fact and the small slope of the wave shows that the reduction of the $\text{Ni}(\text{H}_2\text{O})_6^{++}$ ion is very irreversible. When thiocyanate is added to the solution to form the thiocyanate complex (probably $\text{Ni}(\text{CNS})_4^-$), the half-wave potential is *decreased* to -0.68 volt, and the slope of the wave becomes much steeper, indicating a fairly reversible reduction (14). Similar results were obtained by Lingane and Kerlinger (14) when pyridine was added to a nickel solution to form the pyridine complex.

A similar behavior was noted by Brdička (2) in the case of cobalt. He found that pink solutions of cobaltous chloride, in which the cobalt is present chiefly as $\text{Co}(\text{H}_2\text{O})_6^{++}$, are reduced very irreversibly. When a large excess of chloride ($5 M$ calcium chloride) was added to form the blue chloro complex ions, the reduction potential was shifted by about 0.3 volt to a more *positive* value, and the increased slope of the wave indicated a more reversible reduction.

The author has observed this same effect in the reduction of indium ions. From perchlorate solutions the reduction of the hexaquo indium ion, $\text{In}(\text{H}_2\text{O})_6^{+++}$, is irreversible and requires a large overvoltage, but the reduction of the chloro indium complex ions, formed by adding excess chloride to the solution, is reversible (12).

Strubl (20) has shown that the shape of the composite cathodic-anodic wave of a mixture of simple titanic and titanous ions is like curve 4 in figure 4; the cathodic and anodic reactions are both irreversible. However, the complex ions formed by adding citrate or tartrate to the titanic-titanous solution are reversibly reduced and oxidized, and the shape of the composite wave of the complexes is like curve 2 in figure 4.

The magnitude and direction of the shift of the half-wave potential with temperature is often a very useful criterion of the reversibility of a given electrode reaction, and it can be used to supplement the tests for reversibility that have been described above. When an electrode reaction is irreversible, $E_{1/2}$ is usually (although not invariably) shifted to a more positive value with increasing temperature (easier reduction), and the magnitude of the temperature coefficient is several times larger than that

of a reversible reaction. This effect has been observed by Brdička (2) and by Mr. H. Kerlinger and the author, in the irreversible reduction of the $\text{Co}(\text{H}_2\text{O})_6^{++}$ and $\text{Ni}(\text{H}_2\text{O})_6^{++}$ ions. The excessively large temperature coefficient is related to the activation energy of the irreversible step in the electrode reaction.

These examples illustrate the caution that must be observed in the interpretation of the waves of certain complex metal ions. The tests described in the preceding sections must always be applied to establish the reversibility of the electrode reaction in any given case before quantitative conclusions can be drawn regarding the formula and dissociation constant of the complex.

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SUBSTITUTION AND ORIENTATION IN THE BENZENE RING

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The purpose of this paper is to summarize recent investigations of the mechanism of substitution reactions in benzenoid aromatic compounds and to present an interpretation of the nature of the influence exerted by substituent groups on the course of these reactions. In the latter connection, a method for the precise prediction of the orienting effect of a large variety of substituent groups is described, the basis for which is the accurate evaluation of the relative electrostatic influence of the various groups in polarizing the double bonds of the aromatic ring.

I. THE STRUCTURE OF BENZENE

In spite of the great number of formulas proposed for benzene, each with unique structural features to account for its "aromatic" properties,¹ the structural formula proposed by Kekulé for benzene has become more and more firmly established. In fact, it is now generally accepted that the many characteristic reactions, as well as the unusual stability of the aromatic nucleus, are simply manifestations of such a cyclic conjugated system of double bonds.

In this connection, several authors (13, 60) have recently pointed out that the compound prepared by Willstätter (66, 67), supposedly cyclooctatetraene, actually had chemical and physical properties closely resembling

¹For references and critical discussion see L. F. Fieser in *Organic Chemistry*, edited by H. Gilman, Volume I, pp. 52-149, John Wiley and Sons, Inc., New York (1938).

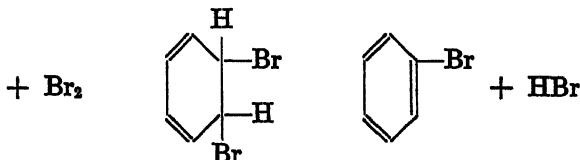
those of an isomer, styrene. Thus, the experimental evidence cited as one of the chief arguments against the "aromaticity" of a cyclic conjugated system of double bonds may be entirely erroneous.

The Kekulé formula in its resonant forms will therefore be used as the basis for the present discussion. Indeed, the facility with which the various experimental facts may be interpreted on this basis affords still further indication as to the validity of this formula.

II. SUBSTITUTION REACTIONS

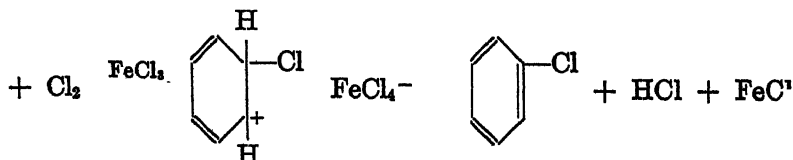
A. Halogenation

Since many of the reagents which will *substitute* in benzene will also *add* to olefins, and since, in the Kekulé formula, benzene is written with double bonds, it has frequently been postulated that the latter process is an intermediate in the former. Thus, for instance, the bromination of benzene has been represented by Holleman (18) and others as addition of bromine, followed by elimination of hydrogen bromide.

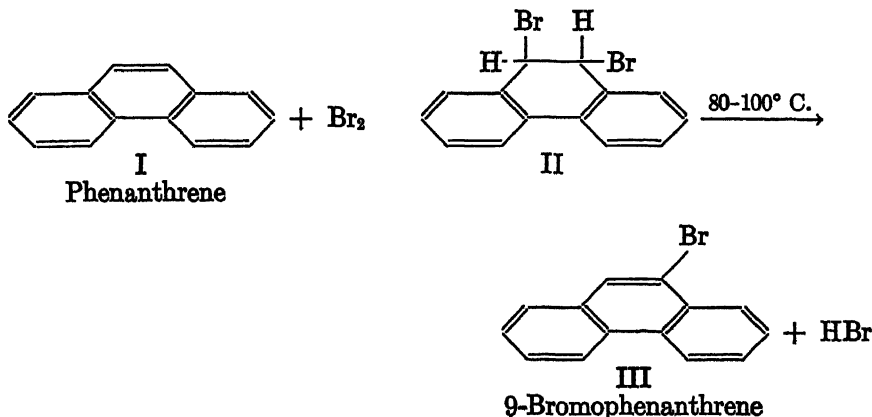


Prins (48) suggested that only one atom of the benzene ring could be involved rather than two, as in addition to a double bond, since the dihydrobenzenoid intermediate above should be too readily attacked further by the substituting reagent. van Alphen (57), however, found that 4,5-dihydrophthalic acid yielded phthalic acid derivatives on treatment with various substituting reagents, invalidating this particular objection to the addition-elimination theory.

In the same year, Pfeiffer and Wizinger (39), on the basis of the colored ionic intermediates formed in the catalytic addition and substitution reactions of the double bond in unsymmetrical diaryl ethylene derivatives, suggested a mechanism for aromatic substitution involving the intermediate formation of an ionic complex. In the case of halogenation, the purpose of a halogen carrier (A), such as ferric chloride, iodine, antimony pentachloride, etc., is to promote the polarization of the halogen molecule and thus the formation of the ionic intermediate.

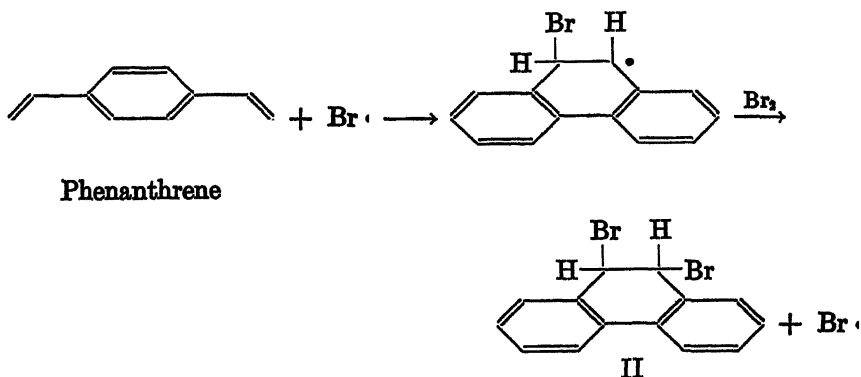


Both the mechanism involving addition followed by elimination, as suggested by Hollemann, and that involving an ionic intermediate, as suggested by Pfeiffer and Wizinger, were, however, supported chiefly by analogy rather than by any direct experimental evidence. For example, it has never been possible to isolate or detect the presence of such dihydro-benzenoid derivatives as those postulated as intermediates in the addition-elimination mechanism for the case of substitution in the benzene ring. With phenanthrene, however, the reaction can actually proceed by such a mechanism. Bromine will add to form a dibromide which is stable at room temperature but which, on warming, loses hydrogen bromide to yield 9-bromophenanthrene. This reaction has, therefore, frequently been considered as evidence for the addition-elimination hypothesis.

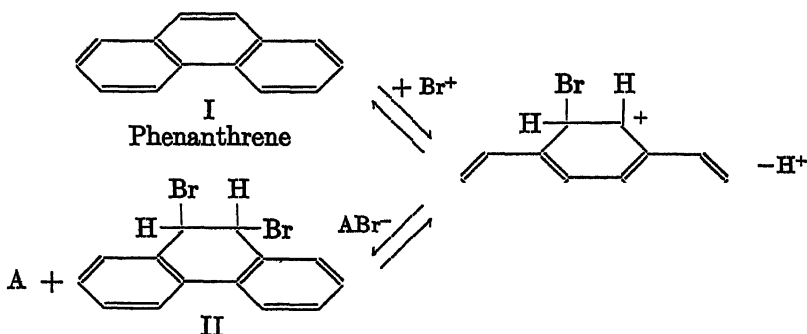
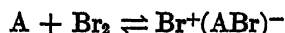


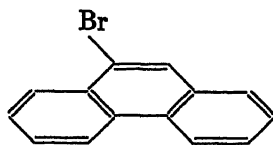
On this account a study of the bromination of phenanthrene offered an excellent opportunity for the determination of the mechanism of substitution. A detailed investigation of this reaction (10, 40, 41, 44), with particular regard to the effect of halogenation catalysts, has shown that the catalytic substitution does *not* proceed according to this addition-elimination scheme, but that the experimental facts are in complete accord with the ionic mechanism suggested by Pfeiffer and Wizinger.

In the first place, it was found that, at 25°C. in carbon tetrachloride solution with no halogenation catalysts present, only the addition reaction occurred and that it proceeded to a measurable equilibrium by a chain mechanism which was entirely light-catalyzed (24, 44). The chain initiating step in the photochemical addition of bromine to a double bond in a non-polar solvent has been found to be the photochemical dissociation of bromine into bromine atoms (2, 33, 52, 61). These active atoms then initiate a chain reaction leading to the addition of bromine to the double bond.



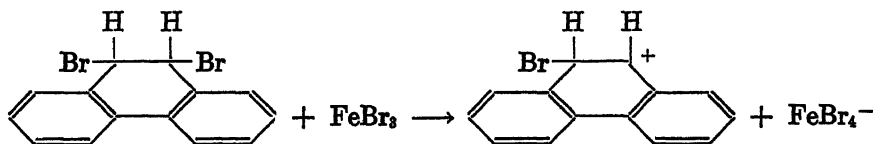
In the presence of a halogen carrier such as iodine or ferric chloride, the reaction of phenanthrene with bromine followed a different course (41). It proceeded smoothly in the dark and was accompanied by the evolution of hydrogen bromide and the formation of 9-bromophenanthrene. The effect of these catalysts could not have been simply the elimination of hydrogen bromide from the dibromide (II), since, under equivalent conditions, the rate of formation of 9-bromophenanthrene (III) from phenanthrene was very nearly the same as from the dibromide and both reactions proceeded at about the same rate as establishment of equilibrium for the addition reaction. These facts would indicate that the reactions were all proceeding through a common intermediate. This intermediate could not be the free radical of the photochemical addition, since iodine, an effective bromination catalyst, strongly inhibits the photochemical addition of bromine to phenanthrene. Since, however, iodine does slowly catalyze the addition reaction in the dark, the reaction in the latter case must be ionic rather than atomic. Allowing A to represent the halogen carrier (iodine, ferric chloride, antimony pentachloride, etc.), the catalytic reaction in the dark may be represented as follows:



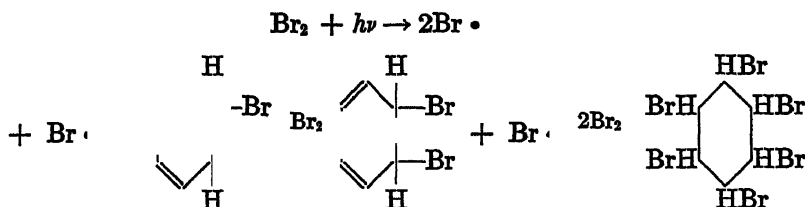


III
9-Bromophenanthrene

The initial step in the reaction from the dibromide (II) involves ionization of a carbon-bromine bond. Since the bond is to a secondary carbon atom alpha to a benzene ring, the reaction could certainly proceed by such a mechanism.² It has frequently been suggested that such reactions, which usually follow first-order kinetics in a polar solvent such as alcohol, are only pseudo-unimolecular and actually involve attack by the solvent molecule. The reaction cannot have been unimolecular in the case under consideration, in which a non-polar solvent, carbon tetrachloride, was used, since the pure dibromide was stable in this solvent, neither dissociating to phenanthrene and bromine nor decomposing to 9-bromophenanthrene and hydrogen bromide. It was only after the addition of a halogen carrier that both reactions occurred simultaneously. The initial step must be electrophilic attack by the catalyst on the bromine atom, a step similar to the polarization of a halogen molecule by the catalyst.



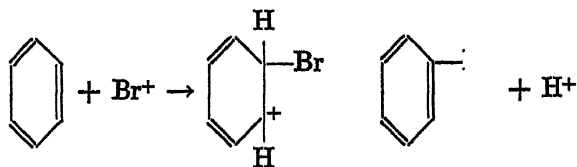
Of these three reactions of phenanthrene with bromine, i.e., the light-catalyzed atomic addition and the chemically-catalyzed ionic addition and substitution, two may be realized for benzene. It is a well-known fact that chlorine and bromine in an inert solvent will add to benzene photochemically to form the corresponding hexahalocyclohexanes (see, for example, 16 and 58).



The catalytic substitution reaction with benzene must proceed just as with phenanthrene, except that the ionic intermediate evidently may be

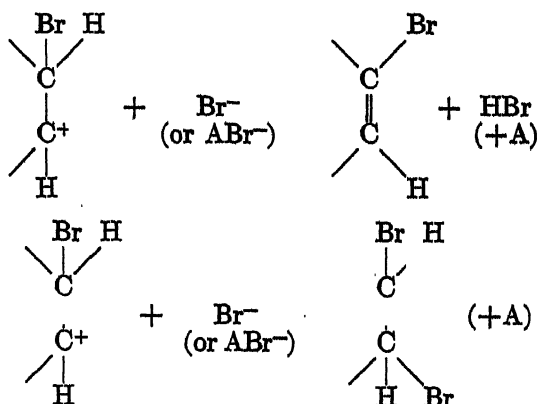
²For references and discussion see reference 14, pp. 131-183.

stabilized only by the loss of a proton, since no addition product is formed during substitution.



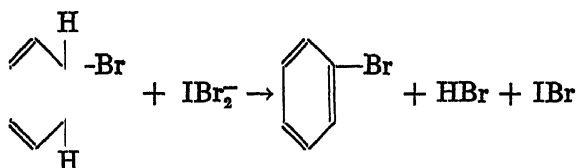
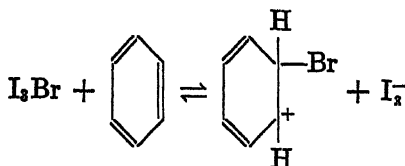
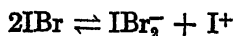
In fact, such elimination of a proton from this type of intermediate, leading to substitution, may be considered a characteristic of the aromatic nature of a substance. Olefinic character, on the other hand, is typified by the acceptance of an anion by such an intermediate, leading to addition. The double bonds of the benzene ring are thus wholly aromatic, while the 9,10-double bond of phenanthrene is only partially so.

Whether the reaction of ionic intermediates of this type will proceed by the acceptance of an anion, leading to addition, or by the loss of a proton, leading to substitution, must depend chiefly on the relative energy relationships for the two courses, i.e., the relative ease with which the bromide (or complex bromide) ion may act as a base and remove a proton from the active intermediate, acting as an acid. The basicity of the bromide ion will be largely dependent on the solvent medium, while the acidity of the intermediate will depend on its structure.



The identity of the mechanism of the iodine-catalyzed substitution in the two hydrocarbons, benzene and phenanthrene, is established by the kinetics of the two reactions since, in each case, the rate of reaction was found to be proportional to the three-halves power of the bromine concentration and to the five-halves power of the iodine concentration (44).

A reaction of such high order must be due to some sequence of reactions involving the two halogens. A series of reactions capable of accounting for the observed kinetics follows:



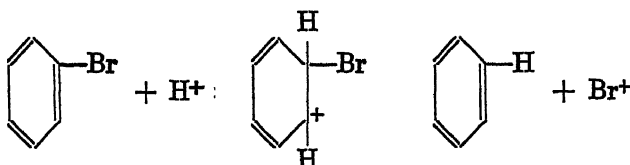
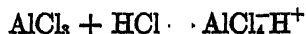
If the rate-controlling step in this series of reactions is the last one, the rate of formation of bromobenzene may be expressed as

$$\frac{d[\text{C}_6\text{H}_5\text{Br}]}{dt} = k[\text{C}_6\text{H}_5\text{Br}^+][\text{IBr}_2^-]$$

Since $[\text{C}_6\text{H}_5\text{Br}^+]$ is proportional to $[\text{I}_3\text{Br}][\text{C}_6\text{H}_6]$, which in turn is proportional to $[\text{I}_2]^{3/2} [\text{Br}_2]^{1/2} [\text{C}_6\text{H}_6]$, and since $[\text{IBr}_2^-]$ is proportional to $[\text{I}_2][\text{Br}_2]$, the expression becomes

$$\frac{d[\text{C}_6\text{H}_5\text{Br}]}{dt} = k^2[\text{C}_6\text{H}_6][\text{Br}_2]^{3/2}[\text{I}_2]^{5/2}$$

The last step in the process is written as irreversible, since hydrogen bromide is not ionized in a non-polar solvent. In the presence of a strong acid, this step too becomes reversible. For instance, the removal and migration of bromine in certain instances of the Friedel-Crafts reaction (7) can be accounted for in the following manner:



Since the bromine cation liberated in this step would be an active brominating agent, it might enter another molecule or a different position in the same molecule, either in the aromatic ring or in an aliphatic side chain. The wandering of bromine from the ring to the side chain, which has been observed during the chlorination of bromotoluenes (1), may be accounted for in a similar manner.

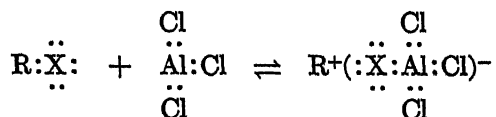


The second step in the course of such a replacement of bromine by hydrogen is the same as the second step for the reversal of the phenanthrene-bromine addition reaction in the presence of a polar catalyst such as iodine.

B. The Friedel-Crafts reaction

Another substitution reaction of aromatic compounds which has been studied extensively with regard to its mechanism is the Friedel-Crafts type of reaction, including acylation with acid chlorides, anhydrides, or esters, and alkylation with alkyl halides, alcohols, ethers, esters, or olefins. Such compounds as boron, aluminum, or iron halides, as well as sulfuric, phosphoric, or hydrofluoric acids, have served as the most useful catalysts for the condensations.

In these cases, also, the experimental evidence indicates an ionic type of mechanism. Wertyporoch and Firla (62) have clearly demonstrated by conductance studies the formation of an ionic complex between aluminum chloride and an alkyl halide.

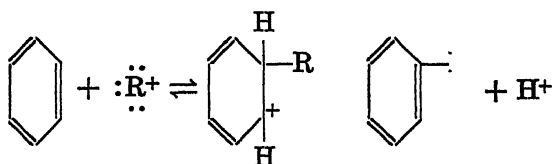
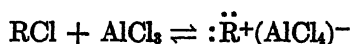


That catalysts for the Friedel-Crafts reaction actually do promote dissociation of the carbon-halogen bond in some manner is supported by an investigation of Bodendorf and Bohme (3). These authors found that optically active α -phenylethyl chloride ($\text{C}_6\text{H}_5\text{CHClCH}_3$) was racemized by the action of various metal salts which act as catalysts for the Friedel-Crafts type of reaction and that activity for the two reactions was roughly parallel:

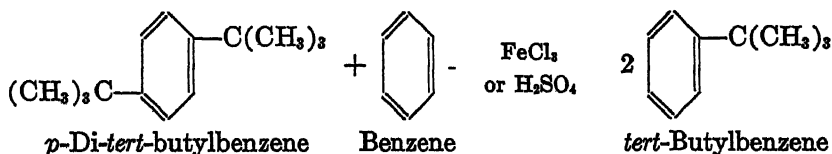


Lithium, silicon, tetramethylammonium, and arsenic chlorides, as well as hydrogen chloride, were ineffective as racemizing catalysts. Ulich and Heyne (56) have measured the equilibrium for the formation of certain of the catalyst-alkyl halide complexes postulated by Wertyporoch and, in addition, found that the rate of alkylation of benzene was directly proportional to the concentration of this catalyst-alkyl halide complex.

The reaction is then entirely analogous to halogenation, the electron-deficient carbonium ion (R^+), which may be either an alkyl or acyl group, reacting just as does the bromine cation (Br^+) to complete its octet by association with a pair of electrons from a double bond of the aromatic nucleus (9, 19, 45).

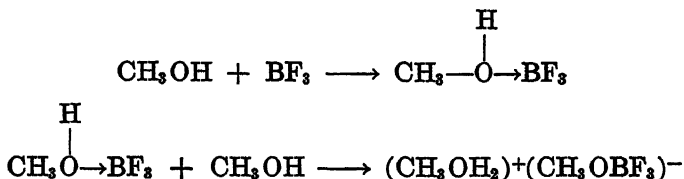


There have been many experimental verifications of the reversibility of the alkylation reaction. Ipatieff and Corson (21) have found that *p*-di-*tert*-butylbenzene in benzene solution is readily converted to *tert*-butylbenzene in the presence of such catalysts as ferric chloride, sulfuric acid, or phosphoric acid.

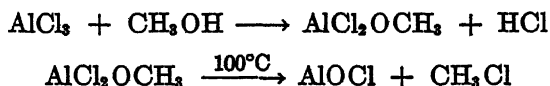


Ipatieff and Corson found that these catalysts were effective only in removing tertiary alkyl groups. It has long been recognized, however, that more vigorous catalysis with aluminum chloride is effective for any type of alkyl group (4, 32; see also 68).

The relative ease of the two possible reactions for the active intermediate in the alkylation, i.e., loss of a proton to yield the alkylated derivative or loss of an alkyl cation to revert to the starting material, must depend on the tenacity with which the group R will retain the electrons it shares with the carbon atom of the ring. The fact that tertiary alkyl groups are most easily removed and primary least is in agreement with this prediction, since the order of electronegativity for alkyl groups has been found to be primary > secondary > tertiary (6, 64). Further evidence that this is

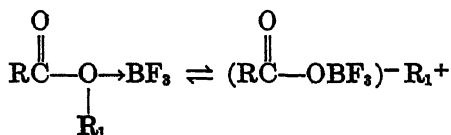


In the case of alcohols as well as ethers, the fluorides differ from the chlorides. While boron fluoride forms dialcoholates which, in the case of primary alcohols, are stable enough to be distilled (30), both boron and aluminum chlorides are vigorously decomposed by alcohols. In fact, the latter reaction has been recommended for the preparation of methyl, ethyl, and propyl chlorides (37).

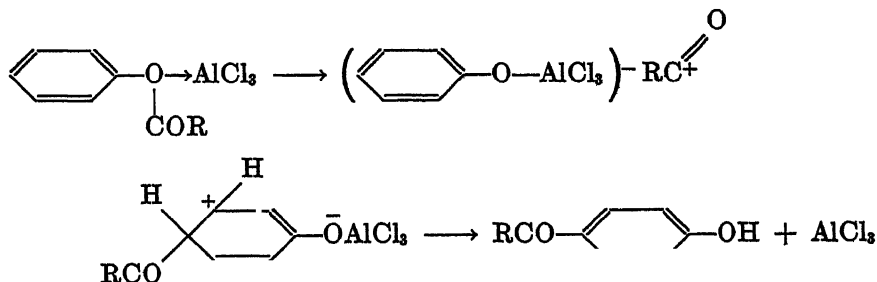


In the reactions of alcohols larger than propyl with aluminum chloride (37, 65) and of secondary alcohols with boron fluoride (46, 65), however, the principal product consists of polymeric material.

A particularly interesting case of the Friedel-Crafts reaction involving oxygen-containing compounds is that of esters. Since an alkyl group has considerably less affinity for electrons than an acyl group, it should be expected that ionization (or polarization) of the alkyl group as a cation would be predominant.

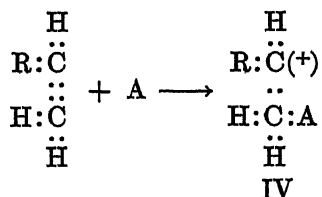


As would be expected on this basis, the reaction of esters in which R_1 is an alkyl group leads principally to alkylation (5, 29, 36). An aryl group, however, would be much less readily released as a cation than an alkyl group (6, 64). In the case of esters of phenols, therefore, it is to be expected that the acyl group might be liberated more readily. This may then recombine with a carbon in the phenol ring.

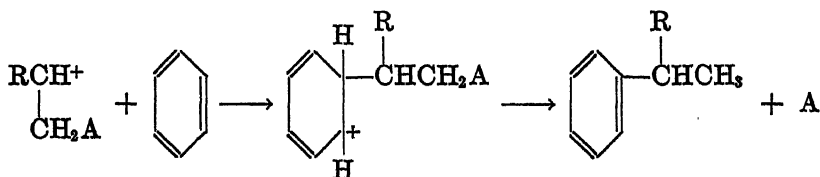


That the acyl group in the ester actually acts as a free acylating agent in the Fries rearrangement is indicated by the fact that Cox (8), in carrying out such a reaction in diphenyl ether, obtained the *p*-acyldiphenylether as well as the rearrangement product.

The condensation of olefins with aromatic compounds to yield alkyl derivatives has also been accounted for on the basis of a cationoid mechanism (19, 45). The electron-deficient catalyst in this case associates with a pair of electrons from the double bond of the olefin to give an active intermediate which is similar to that of the ordinary Friedel-Crafts reaction, in that one carbon atom has only a sextet of electrons.



This carbon atom may then complete its octet by association with an electron pair from the aromatic nucleus. The final step is elimination of the catalyst, followed by the α, γ -shift of a proton.

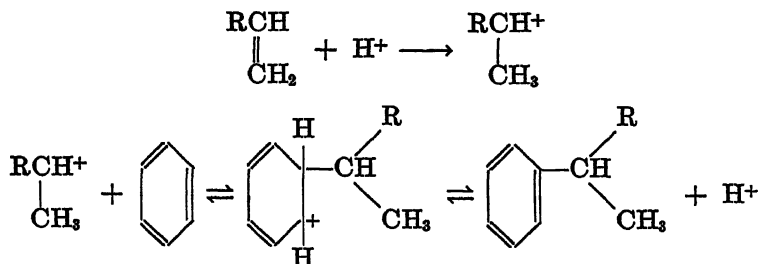


Completion of the octet of the electron-deficient carbon atom of IV by association with an electron pair of a second olefin molecule will, of course, result in polymerization (19).

A necessary corollary to such an intermediate complex formation for the olefins should be their *cis-trans* isomerization by these catalysts. This has been established for boron fluoride acting on *cis*-stilbene (47) and for the other Friedel-Crafts catalysts acting on diethyl maleate (11). In the latter case, the effectiveness of the catalysts in isomerization was closely parallel to that in condensation.

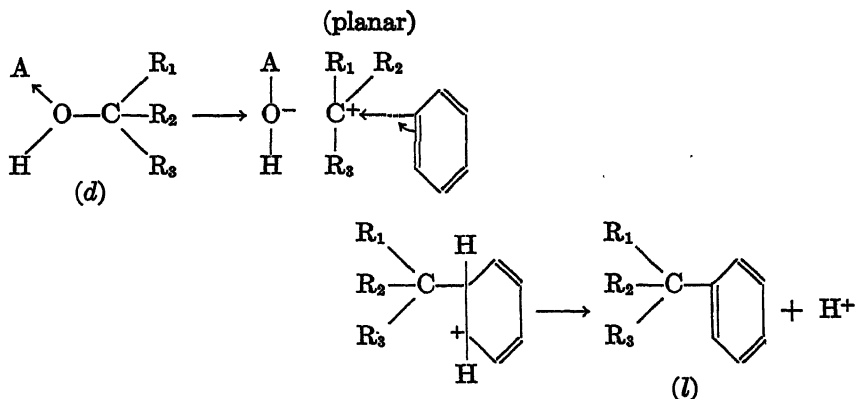


In the case of catalysis of any of these reactions by strong acids, such as sulfuric, phosphoric, or hydrofluoric acid or hydrogen chloride in the presence of aluminum chloride, the electron-deficient catalyst is evidently a proton.



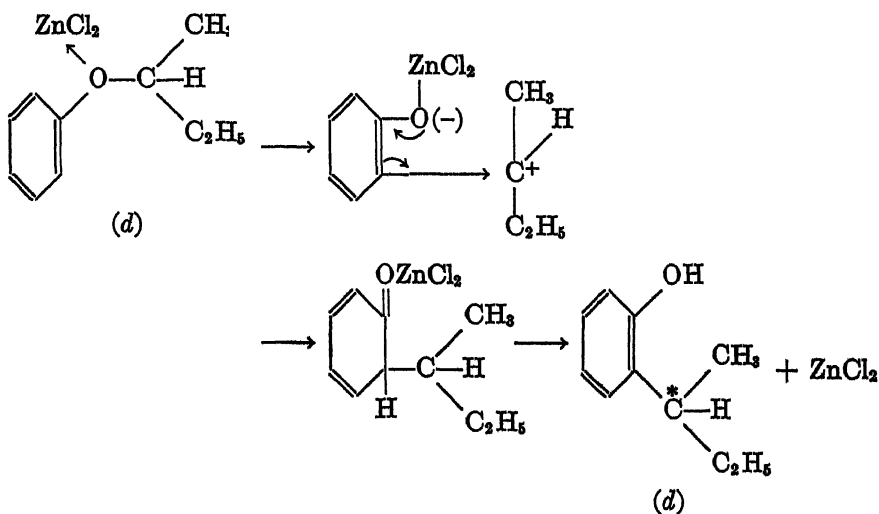
In accord with the views of Whitmore on molecular rearrangements (63), the electron-deficient carbon atom in the intermediate alkyl cation should tend to be secondary rather than primary, and tertiary rather than secondary. This tendency must account for the rearrangements of the alkyl group which are frequently encountered in Friedel-Crafts reactions (see, e.g., reference 22).

In those cases in which a possible rearrangement does not occur, such as alkylation using alcohols with aluminum chloride (20) or with boron fluoride (28) as catalysts, the existence of the alkyl cation may be too transient to allow for the migration of a proton (or an alkyl group), leading to a rearrangement product. This must certainly be true in the particular case of the boron-fluoride-catalyzed alkylation by means of alcohols, since *d*-*sec*-butyl alcohol was found to yield *l*-*sec*-butylbenzene (46). The rotation of the hydrocarbon ($[\gamma]_D^{20} = -0.16$) indicates not only an inversion of the configuration of the asymmetric carbon atom but also simultaneous racemization to the extent of about 95 per cent (27). This is the characteristic behavior of those replacement reactions at a saturated asymmetric carbon atom in which the rate-controlling step is the primary dissociation of the reagent into ions (reference 14, pages 131-183). If the asymmetric alkyl cation reacts almost simultaneously with the process of ionization, the only avenue of approach is at the face opposite that being vacated by the anion. The process will then result in almost complete inversion of configuration. The longer the life of the cation, however, the more

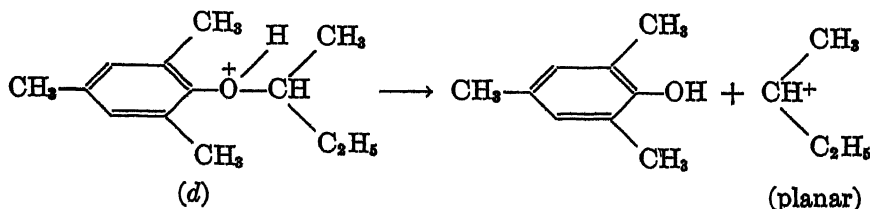


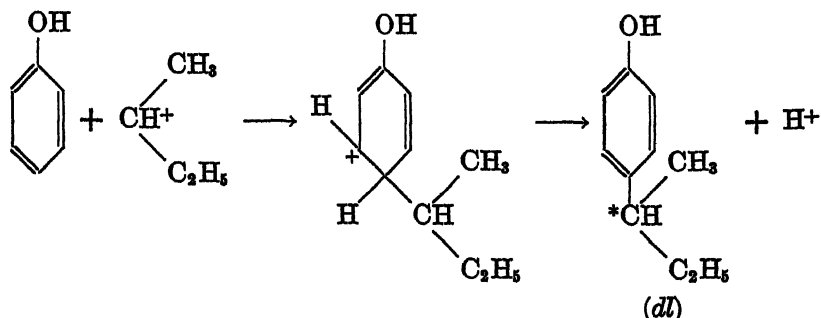
extensive will be its racemization before subsequent reaction. In the case of the alkylation of benzene, using an alcohol with boron fluoride as catalyst, the alkyl cation must have had a free enough existence to racemize considerably. It did, however, react so rapidly that inversion of configuration was a significant reaction.

It is of particular interest to compare these results of alkylation, using *d*-*sec*-butyl alcohol, with the results of Sprung and Wallis (55) on the rearrangement of *d*-*sec*-butyl aryl ethers. In each case the reaction involves scission of a carbon-oxygen bond, followed by union of the alkyl group thus liberated with the carbon atom of a benzene ring. In the case of the rearrangement, however, when the alkyl group moved to the ortho position in the same molecule, the reaction apparently proceeded with retention of configuration, accompanied by racemization. This may be readily accounted for on the basis that the planar intermediate alkyl cation never leaves the sphere of influence of the anion.



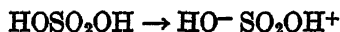
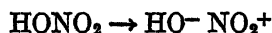
This course for the reaction is well substantiated by the fact that, when the alkyl group must react with a second molecule, as in the case of the alkylation of phenol with *d*-*sec*-butyl mesityl ether (12), the *sec*-butylphenol produced is racemic.



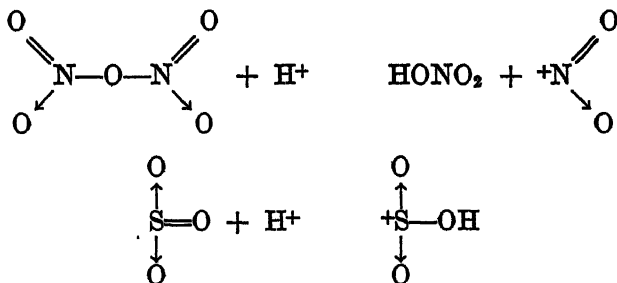


C. Nitration and sulfonation

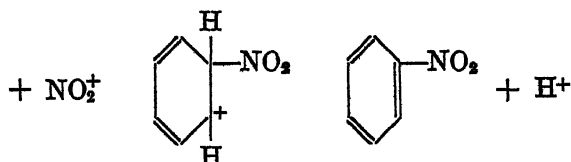
Although experimental data for the interpretation of the mechanism of nitration and sulfonation are not available, both reactions have been accounted for on the basis of a cationoid mechanism similar to that which has been established for the cases of halogenation and alkylation or acylation.¹ During the process, the nitric and sulfuric acids must dissociate in such a manner as to lose a hydroxyl group. It has been postulated that this occurs so as to leave the nitro and sulfonic acid groups as cations.



These active intermediate cations may be formed by interaction of the respective acid anhydrides with a proton



The electron-deficient cations then react by picking up a pair of electrons from a double bond of the aromatic ring, and a proton is lost from the intermediate thus formed.



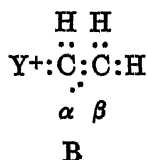
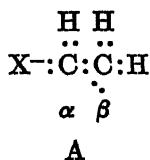
III. ORIENTATION

If the cationoid mechanism for substitution as presented above is to be considered entirely satisfactory, it should be capable of accounting for the influence of substituent groups in directing the entering group to certain preferred positions in the ring, as well as in affecting the ease with which the substitution can occur.

For those reactions proceeding by the cationoid mechanism,³ the entering group in each case associates with a pair of electrons from a double bond of the aromatic nucleus. The substituent already present may therefore orient the incoming group by influencing the position of these electrons. The substituent may further influence the reaction (independently of its orienting effect) by conditioning the availability of these electrons, i.e. it may facilitate or hinder further substitution.

There are many manifestations of the effect of various substituent groups at a double bond on the position of the electrons of the bond. In particular, the mode of addition of most unsymmetrical reagents to a double bond is governed by the nature of the polarization of the bond imposed by the various neighboring groups.

If, for instance, the over-all effect of a group were negative, the electrons of the double bond, *particularly the extra labile pair*, would be repelled. This has been represented below in formula A, the α -carbon atom acquiring some measure of positive charge while the β -carbon atom becomes correspondingly negative. The second case (B), in which a group has a positive effect, will result in polarization of the double bond in the opposite sense.

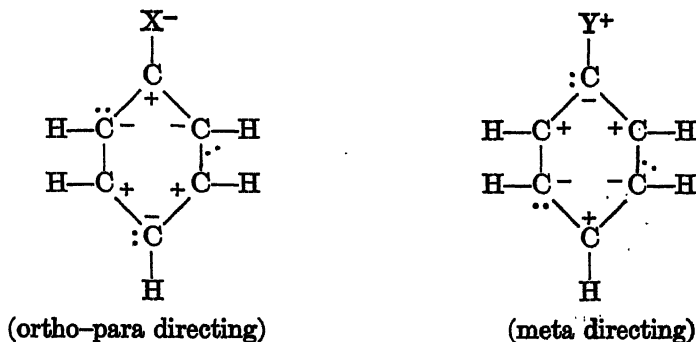


Addition of a reagent H^{+}A^{-} to these double bonds will then, of course, occur in such a manner that the negative portion of the adding reagent

³There are a few reactions, proceeding by different mechanisms, which do not obey the ordinary rules for orientation. For those involving the reaction of an anion, such as the replacement of chlorine by hydroxyl ion (see Waters: *Physical Aspects of Organic Chemistry*, pp. 453-459, Routledge, London (1935)), the influence of substituent groups, both as to relative ease of replacement and relative position of activation, is opposite to that for the usual cationoid substitution reactions. This is as would be expected according to the mechanism for orientation outlined herein. Those reactions proceeding through free radical intermediates (Hey and Waters: *Chem. Rev.* **21**, 169 (1937)) lead chiefly to ortho and para derivatives, regardless of the nature of the substituent group present. Thus, on the basis of orientation, mercuration and metalation reactions may belong in the latter class.

will become attached to the more positively charged carbon atom. This is a statement of Michael's rule (31).

These two cases for a group attached to the conjugated system of double bonds in the aromatic nucleus may then be represented as follows (42):



For each double bond, it is usually the polarity of the atom (or atoms) directly attached to the double bond which is the principal factor. This follows simply from Coulomb's law of electrostatic attraction and repulsion, the interaction of two charges decreasing in proportion to the square of the distance between them.⁴

Thus the group X, having a negative field with respect to the electrons of the ring, will shift the electrons in such a way as to increase the electron density at the ortho and para positions. Since the group which substitutes in the benzene ring is an electron-deficient group, tending to pick up a pair of electrons from the aromatic nucleus, it may obviously do so most readily at the ortho and para positions in this case. The group X will, therefore, be an ortho-para-directing group.

The group Y, with the opposite effect, will shift the electrons in the opposite direction, the electron density being greater in the meta positions. The entering group will thus tend to associate with the carbon atoms in the meta positions in this case. The group Y will therefore be a meta-directing group.

A. Ease of substitution

The ease of cationoid substitution in an aromatic compound will be governed by the ease of the initial attack involving acquisition of a pair of electrons from the ring. A substituent which will increase the electron density will increase the ease of substitution. One means of affecting the relative electron densities of the atoms in the ring must be the polarization of the bonds in the ring, induced by the substituent in the manner outlined above.

⁴For the interatomic distances dealt with in this paper, the dielectric constant has been assigned the value of unity.

In addition, the substituent may evidently affect the over-all electron density of the ring by donating or withdrawing electrons. For example, the chlorine atom acquires a major share of the pair of electrons it shares with the ring, thus decreasing the over-all electron density of the ring. Chlorobenzene is thus more resistant to cationoid substitution than benzene.

Furthermore, it seems evident that the atom attached to the ring (referred to as the α -atom in table 2) may also share with the ring a *portion* of the charge which it acquires from some other atom or atoms. For example, the oxygen of the hydroxyl group will acquire a somewhat greater share in the electrons binding it to the ring than will the carbon atom to which it is bound. On this basis the hydroxyl group might resemble the chlorine atom and deactivate the ring to further substitution. The oxygen atom of the hydroxyl group, however, differs from the chlorine atom in that a major portion of its negative charge is acquired from the hydrogen atom. It apparently may share a portion of this negative charge acquired from the hydrogen with the ring, thus accounting for the fact that the hydroxyl group increases the ease of aromatic substitution.

Similarly, in the case of such groups as the carboxyl and trichloromethyl groups, further substitution in the ring must be hindered by the fact that a portion of the positive charge on the carbon atom in these two groups, acquired from the oxygen and chlorine atoms, respectively, is shared with the ring.

B. Rules of orientation

Many of the rules which have been suggested for predicting the orienting effect of a group substituted on the benzene ring, and particularly the more modern rules, such as those proposed by Hammick and Illingworth (17) and by Latimer and Porter (26), may actually be interpreted as empirical approximations for determining whether, in a substituent group, the atom which is directly attached to the ring is relatively positive or negative.

Since the over-all effect of many simple groups will be governed principally by the charge on this atom, which is closest to the electrons affected, the rules will correctly predict the orienting influence for these simple groups.

In order to determine whether the orienting influence of substituent groups could be more accurately correlated with their electrostatic effect, the charge on all the atoms of the substituent group should be taken into account, as well as their position relative to the electrons affected. This calculation has been carried out, using accurate molecular models, with charges assigned to the various atoms on the basis of bond moments determined from experimental dipole moment data (43). Many of these in-

dividual bond moments have already been estimated (53, 54). Others have been calculated in an analogous manner from more recent dipole moment data on saturated aliphatic derivatives containing the bond in question and from the respective atomic radii. These various individual bond moments are summarized in table 1.

TABLE 1
Individual bond moments and atomic charges in fractions of an electron

BOND	μ (BOND MOMENT)	d (BOND LENGTH)	Q (ATOMIC CHARGE) $= \mu \times 10^{-18} / d \times 10^{-8}$ E.S.U.	$Q/4.77 \times 10^{-10}$ = ATOMIC CHARGE IN FRACTIONS OF AN ELECTRON
	Debyes	Å.		
(+) (-)				
H—C	0.30	1.08	0.28×10^{-10}	0.06
C—O	0.85	1.43	0.60	0.13
C=O	2.40	1.24	1.93	0.40
C—S	0.95	1.81	0.53	0.11
C—N	0.40	1.47	0.27	0.06
C≡N	3.60	1.15	3.13	0.66
C—F*	1.60	1.44	1.11	0.23
C—F (3F)†	1.20	1.44	0.83	0.17
C—Cl	1.70	1.76	0.97	0.20
C—Cl (2 Cl)†	1.2	1.76	0.68	0.14
C—Cl (3 Cl)	0.85	1.76	0.48	0.10
C—Br	1.70	1.91	0.89	0.19
C—I	1.60	2.09	0.77	0.16
H—O	1.53	0.97	1.58	0.33
H—N	1.31	0.91	1.44	0.30
H—S	0.68	1.35	0.50	0.11
N→O	3.2	1.36	2.35	0.49
N=O	2.0	1.18	1.70	0.36
S—O	(0.25?)	1.70	0.15	0.03
S→O	2.5	1.70	1.47	0.31

* The values for the carbon-halogen bonds were calculated from primary halides, rather than from methyl halides.

† See Smyth and McAlpine: *J. Chem. Phys.* 1, 190 (1933).

The charge on each atom associated with these bond moments was calculated simply by dividing the bond moment by the bond length. The basic charge distribution for the molecules was then assumed to be that derived from the various bonds for each atom. These charges, although undoubtedly modified by dipolar interaction, nevertheless formed a satisfactory basis for the calculations.

The effect of any particular atom on an electron of the adjacent double bond is then proportional to its charge divided by the square of its dis-

tance (r) from the electrons. That portion of this total force on the electrons effective in polarizing the double bond equals the total force multiplied by the cosine of the angle (θ) formed between the line connecting the electrons with the atom in question and the bond itself. This force, referred to as the polarizing force, is calculated for each particular atom (x) from the following expression,

$$\text{Polarizing force} = \frac{4.77 \times 10^{-10} \times e_x}{r^2} \cos \theta$$

in which 4.77×10^{-10} is the electronic charge in electrostatic units and e_x is the charge on the atom (x) in question.

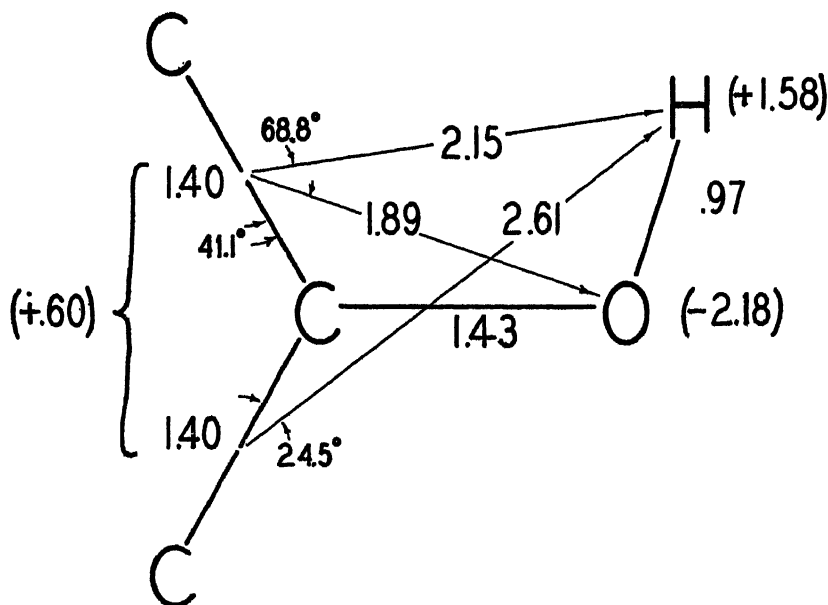


FIG. 1. Model for the hydroxyl group. Distances in Ångström units; charges in e.s.u. $\times 10^{10}$. Polarizing force = $[-2.18 \cos 41.1^\circ/1.89^2 + (1.58 \cos 68.8^\circ/2.15^2 + 1.58 \cos 24.5^\circ/2.61^2)/2] \cdot 4.77 \times 10^{-4} = -1.46$ dynes.

In the calculations, free rotation of substituents⁵ and resonance of the electrons in the aromatic nucleus have been assumed. When an atom could occupy several positions relative to the electrons of the double bond in the ring, the arithmetic mean of the polarizing force in the extreme

⁵ In several cases, such as that of the carbomethoxyl group, complete free rotation was impossible, owing to interference of the ortho hydrogen atoms. In such cases the extreme position of rotation was taken as that in which the groups approached each other as closely as the models would permit.

positions was used. Models for two particular cases, the hydroxyl group and the acetyl group, are illustrated in figures 1 and 2, respectively.

From such models the electrostatic effect of a large number of substituents of known orienting influence has been calculated. The correlation between these two factors, listed in table 2,⁶ is so accurate that the degree of meta orientation may be estimated to within less than ± 10 per cent from the value of the polarizing force.

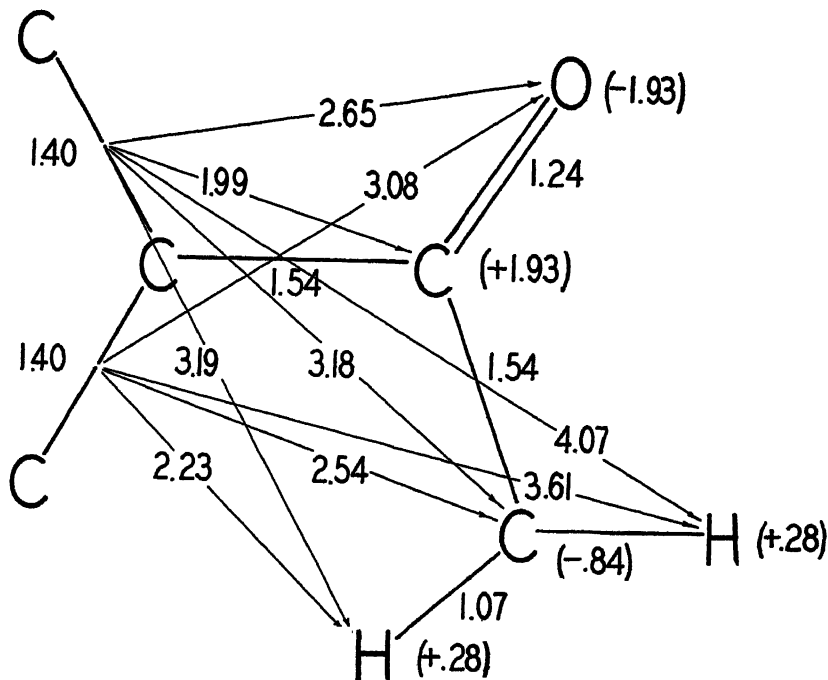


FIG. 2. Model for the acetyl group. Polarizing force = $[1.93 \cos 42.4^\circ/1.99^2 + (-1.93 \cos 69.3^\circ/2.65 - 1.93 \cos 27.7^\circ/3.08^2)/2 + (-0.84 \cos 78.7^\circ/2.54^2 - 0.84 \cos 19.9^\circ/3.18^2)/2 + 3(0.28 \cos 103.3^\circ/2.23^2 + 0.28 \cos 1.0^\circ/3.19^2 + 0.28 \cos 72.8^\circ/3.61^2 + 0.28 \cos 29.8^\circ/4.07^2)/4] \cdot 4.77 \times 10^{-4} = 0.89$ dyne.

It should be noted that the division between the ortho-para-directing and the meta-directing groups falls, as it should, at the value for the hydrogen atom; i.e., a group more negative than hydrogen is predominantly ortho-para directing, while one more positive is meta directing.

No substituents containing an olefinic double bond have been included in table 2, since no method is available for calculating the absolute degree of polarization, and thus the charge distribution, for such bonds. Fur-

⁶The data listed in table 2 should be useful also for comparing various groups in regard to their polarizing effect on a simple olefinic double bond.

TABLE 2

The electrostatic polarizing influence of various substituent groups on an adjacent double bond, compared with their orienting influence

GROUP	CHARGE ON α -ATOM	POLARIZING FORCE	OBSERVED META ORIENTATION ^(a)
	<i>E.S.U.</i>	<i>dynes</i>	<i>per cent</i>
NH ₂	-3.15×10^{-10}	-1.66×10^{-4}	3
OH.....	-2.18	-1.46	
NHCH ₃	-1.98	-1.41	
OCH ₃	-1.20	-1.21	
N(CH ₃) ₂	-0.81	-1.16	
F.....	-1.11	-1.12	
Cl.....	-0.97	-0.68	
C(CH ₃) ₃	0	-0.62	
Br.....	-0.89	-0.55	
I.....	-0.77	-0.39	
CH ₃	-0.84	-0.39	4
COO ⁻	+2.53	-0.07	33 ^(b)
CH ₃ CN.....	-0.56	+0.32	14 ^(c)
CH ₂ Cl.....	+0.41	0.36	4 (11 ^(d))
CH ₂ Br.....	0.33	0.36	7
CH ₂ F.....	0.55	0.39	17
H.....	0.28	0.45	(40)
C(CH ₃) ₂ NO ₂	0.27	0.52	30 ^(e)
CH ₂ NO ₂	-0.29	0.68	50
CHCl ₂	+1.08	0.75	34
N=O.....	1.43	0.77	(1)
CH ₂ NH ₂ ⁺	-0.29	0.80	49
CONH ₂	+2.20	0.84	69
COCH ₃	1.93	0.89	55
CCl ₃	1.44	0.93	64 (100 ^(e))
CHO.....	1.65	0.98	79
CH(CN) ₂	-0.28	1.05	68 ^(e)
CH ₂ N(CH ₃) ₂ ⁺	-0.29	1.07	88
COOCH ₃	+2.53	1.23	73
COOH.....	2.53	1.25	82 (90 ^(h))
SO ₂ CH ₃	1.88	1.36	98 ⁽ⁱ⁾
CF ₃	2.49	1.41	90 ^(j)
COCl.....	2.90	1.73	90
C≡N.....	3.13	1.80	80 (88 ^(e))
SO ₂ H.....	2.56	1.87	72 (95 ^(k))
NO ₂	3.78	2.21	93
NH ₃ ⁺	0.18	2.21	(95 ^(l))
N(CH ₃) ₂ ⁺	3.69	3.21	100

^(a)Unless otherwise noted, these values have been taken from those given by L. F. Fieser in *Organic Chemistry* (edited by H. Gilman), p. 140, John Wiley and Sons,

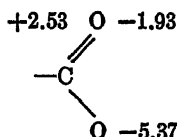
thermore, since the olefinic bond is a reactive center in the molecule, it may well be modified considerably while in contact with a reagent reactive enough to substitute in the aromatic ring.

The values for the percentage of meta orientation are principally those for nitration (footnote 1 (page 37) and reference 50). The more recent data are referred to in the table. These values may vary somewhat under differing experimental conditions or for different reagents. Significantly different values have been indicated in the table.

Although the data presented do not lead to any indication of the actual degree of polarization of the bonds in the aromatic ring, it is significant to compare the electrostatic polarizing forces in table 2 with two recent estimations of the effect of substituents on the charge distribution in the benzene ring (15, 51). If this force is the principal factor involved in polarizing the bonds of the benzene ring, then the difference in charge between adjacent atoms of the ring, such as the meta and para carbon atoms in a benzene derivative, should parallel the electrostatic polarizing force of the substituent.

Inc., New York (1938), and are principally for nitration.

^(b)Smith: J. Chem. Soc. **1934**, 213. The calculation of the polarizing force was based on the ion as indicated with the following charge distribution:



Any hydration of this ion by hydrogen bonding with the solvent (water) would increase the value of the polarizing effect and thus bring this group into agreement with the others in the table.

^(a)Flürscheim and Holmes: J. Chem. Soc. **1928**, 2230.

^(d)Flürscheim and Holmes: J. Chem. Soc. **1928**, 1607.

^(c)Baker and Ingold: J. Chem. Soc. **1926**, 2462.

^(d)Hammick, New, and Sutton (J. Chem. Soc. **1932**, 742) have suggested that the reason the nitroso group directs ortho-para, contrary to predictions, is that nitroso-benzene actually exists in solution as a dimer.

^(e)Lauer: J. prakt. Chem. **142**, 252 (1935).

^(b)Reese: Chem. Rev. **14**, 55 (1934).

^(d)Baldwin and Robinson: J. Chem. Soc. **1932**, 1445.

^(d)Finger, Nachtrieb, and Reed: Trans. Illinois State Acad. Sci. **31**, 132 (1939).

^(d)Lauer: J. prakt. Chem. **143**, 127 (1935).

^(d)Since aniline is a weak base, the ortho and para derivatives frequently obtained from aniline salts may arise from the reaction of free aniline. This view is supported by the fact that increased acid concentration always results in increased meta orientation in aniline and its derivatives (Noelting and Collin: Ber. **17**, 261 (1884); Clemo and Smith: J. Chem. Soc. **1928**, 2414).

Hammett (15) has found that, for a large variety of reactions of benzene derivatives, the effect of a substituent in the meta or para position on the rate (or equilibrium) of the reaction may be expressed as

$$\ln k - \ln k_0 = \rho\sigma \quad (1)$$

where k is the rate constant for the meta- or para-substituted derivative, k_0 is that for the unsubstituted compound, and ρ and σ are two constants. The reaction constant ρ depends solely on the nature of the reaction under consideration, e.g., the hydrolysis of an ester, esterification, the ionization

TABLE 3

Comparison of the difference in the substituent constant σ for a substituent in the meta and para positions with the polarizing force of the substituent (table 2)

GROUP	σ_m^*	σ_p	$\Delta\sigma$	POLARIZING FORCE
NH ₂	-0.161	-0.660	-0.499	-1.66×10^{-4}
OCH ₃	+0.115	-0.268	-0.383	-1.21
F.....	0.337	+0.062	-0.275	-1.12
Cl.....	0.373	0.227	-0.146	-0.68
Br.....	0.381	0.232	-0.149	-0.55
CH ₃	-0.069	-0.170	-0.101	-0.39
I.....	+0.352	+0.276	-0.076	-0.39
COCH ₃	0.306	0.874	+0.568†	+0.89
CHO.....	0.381	1.126	0.745†	0.98
COOH.....	0.355	0.728‡	0.373	1.25
C≡N.....	0.678	1.000‡	0.322	1.80
NO ₂	0.710	1.27‡	0.56	2.21

* The values for σ are taken from reference 14, page 188.

† The only determinations from which these data were calculated were the rates of hydrolysis of the corresponding sulfonic acids.

‡ These data are for phenol derivatives. The evidence indicates that these values may be different for other types of compounds (15).

of an acid, etc., whereas the substituent constant σ is dependent solely on the nature of the substituent in the meta or para position. The substituent constant is interpreted by Hammett as a measure of the electron density produced by a substituent at the reactive group, while ρ measures the susceptibility of a particular reaction of this group to changes in electron density.

A comparison of the difference in values of σ for different substituent groups in the meta and para positions is presented in table 3, accompanied by the polarizing force of the substituents. The close parallel between the polarization as measured by σ and the polarizing force would indicate strongly that these two values must be directly connected.

Ri and Eyring (51) have estimated the charges on each nuclear carbon atom of benzene derivatives by analysis of the data in the literature for the competitive rates of nitration of various derivatives of benzene. They based their calculation on the assumptions that nitration proceeds by a cationoid mechanism and that the interaction between the nitro cation (NO_2^+) and the charge on the carbon atom with which it reacts changes the free energy of activation by the amount $e_y e_n / rD$, where e_y is the charge on the carbon atom undergoing nitration, e_n the charge on the nitro group, r the distance between the two charges in the intermediate activated complex, and D the dielectric constant of the medium separating the charges. The distance r was taken as 1.6\AA ., a value 10 per cent longer than the normal carbon-nitrogen bond. At this distance there can be no molecules separating the charges, so the dielectric constant is considered to be unity. The rates of substitution at a carbon atom in benzene (k_H) and at a carbon atom y in a benzene derivative (k_y) will then be

$$k_H = \alpha e^{-\Delta F/RT} \quad (2)$$

$$k_y = \alpha e^{-(\Delta F + e_y e_n / rD)/RT} \quad (3)$$

from which

$$k_y/k_H = e^{-e_y e_n / rDRT} \quad (4)$$

From the rates of nitration of a benzene derivative in each position relative to the rate for benzene itself, Eyring and Ri were thus able to estimate the charge on each atom in the ring by use of equation 4. The differences between this charge (e_y) for the positions meta and para to the various substituents are summarized in table 4, again compared with the electrostatic polarizing force of the substituent group. The agreement is even more satisfactory than for the values of σ (table 3).

It is of some interest to point out the theoretical relationship and implications of the work of Hammett and of Ri and Eyring as well as that of Jones (23). The latter investigator has found that, in the rate expression

$$k = \alpha e^{-E/RT}$$

for the halogenation of a large variety of aromatic derivatives, particularly of the type $p\text{-XC}_6\text{H}_4\text{OR}$, α remained constant while the effect of the various substituents on the activation energy E was additive. Expressing the rate of chlorination in the same manner as that of nitration (equation 3), the activation energy, E , may be represented as

$$E = \Delta F + e_y e_n / rD \quad (5)$$

where ΔF represents a constant, e_y the charge on a carbon atom y of the nucleus, e_x the charge on the entering substituent, in this case a chlorine cation, Cl^+ , and r and D represent the distance and dielectric constant as before. If the effect of a substituent is principally on the charge, e_y , and if this influence for two groups in the same ring is substantially independent,

TABLE 4

Comparison of the differences between the charges on the meta and para carbon atoms, as calculated by Ri and Eyring (51), with the polarizing force (table 2)

GROUP	e_{ym}	e_{yp}	Δe_y	POLARIZING FORCE
	<i>E.S.U. $\times 10^{10}$</i>	<i>E.S.U. $\times 10^{10}$</i>	<i>E.S.U. $\times 10^{10}$</i>	
F.....	0.0933	0.0035	-0.0898	-1.12×10^{-4}
Cl.....	0.115	0.027	-0.088	-0.68
Br.....	0.116	0.0305	-0.0855	-0.55
I.....	0.0909	0.0065	-0.0844	-0.39
CH_3	-0.0128	-0.0532	-0.0404	-0.39
CH_2Cl	0.114	0.070	-0.044	+0.36
CHCl_2	0.117	0.104	-0.013	0.75
CCl_3	0.0876	0.0892	+0.0016	0.93
COOCH_3	0.0929	0.1230	+0.030	1.23
COOH	0.0411	0.0871	+0.046	1.25
NO_2	0.191	0.248	+0.157	2.21

then the effect of the two substituents on the activation energy for the reaction will be additive.

$$E = \Delta F + \frac{(e_{y_1} + e_{y_2})e_x}{rD} \quad (6)$$

In this expression, e_{y_1} represents the charge induced on the carbon atom y by one substituent, and e_{y_2} that induced by a second substituent. The additivity of the effects of different groups may thus be adequately accounted for by equation 6.

The constancy of the probability factor⁷ α , observed experimentally by Jones and implicit in the derivation of equation 4 from equations 2 and 3 by Ri and Eyring, leads to a direct interpretation of the ρ and σ constants of Hammett (equation 1). If we extend equation 2 to include the possibility that, in the benzene derivative under consideration ($\text{C}_6\text{H}_5\text{R}$), the carbon atom to which the reactive group R is attached may have a charge e_R , the equation can be represented as

$$k_H = \alpha e^{-(\Delta F^0 + e_R e_x / rD) / RT} \quad (7)$$

⁷Hammett (14) has presented a discussion of the conditions necessary for the constancy of α , considered from the viewpoint of the transition-state theory.

A second substituent, y , in this compound will change the value of e_R to e_y .

$$k_y = \alpha e^{-(\Delta F^0 + e_y e_x / rD) / RT} \quad (8)$$

or

$$\ln k_H - \ln \alpha = -(\Delta F^0 / RT + e_R e_x / rD \cdot RT) \quad (9)$$

and

$$\ln k_y - \ln \alpha = -(\Delta F^0 / RT + e_y e_x / rD \cdot RT) \quad (10)$$

Subtracting equation 9 from equation 10, we find

$$\ln k_y - \ln k_H = -(e_y - e_R) e_x / rD \cdot RT \quad (11)$$

The term⁸ $(e_y - e_R)$ is the change in electron density induced on the carbon atom of the benzene adjacent to R by the substituent and is independent of the reaction; thus it corresponds to σ . The term e_x / rD , the charge on the attacking reagent divided by its separation from the point of attack in the activated complex, is dependent solely on the nature of the reaction and thus corresponds to the ρ factor.

The factor $1/T$ is absorbed in the ρ factor. The only case for which ρ has been determined at two different temperatures is for the reaction of aniline derivatives with 2,4-dinitrochlorobenzene at 25° and 100°C. At the lower temperature, $\rho = -3.1910 \pm 0.07$; at the higher, $\rho = -2.581 \pm 0.16$. Multiplying by the absolute temperature, the first value becomes -950 ± 20 and the second -962 ± 60 . A much more thorough test of the inverse proportionality of ρ and the absolute temperature is necessary.

The fact that equation 1 fails for cases in which the substituent is ortho to the reactive group may be accounted for very simply by the failure of the probability or steric factor α to remain constant, although a direct polar influence of the substituent on the reactive group, rather than one passed through the ring, is also undoubtedly of considerable importance.

The wide variety of reactions for which equation 1 may be applied implies the constancy of both α and ΔF^0 for all these reactions. If ΔF^0 actually does remain constant for all these diverse reactions, it can of course be included in α , and the rate expression becomes

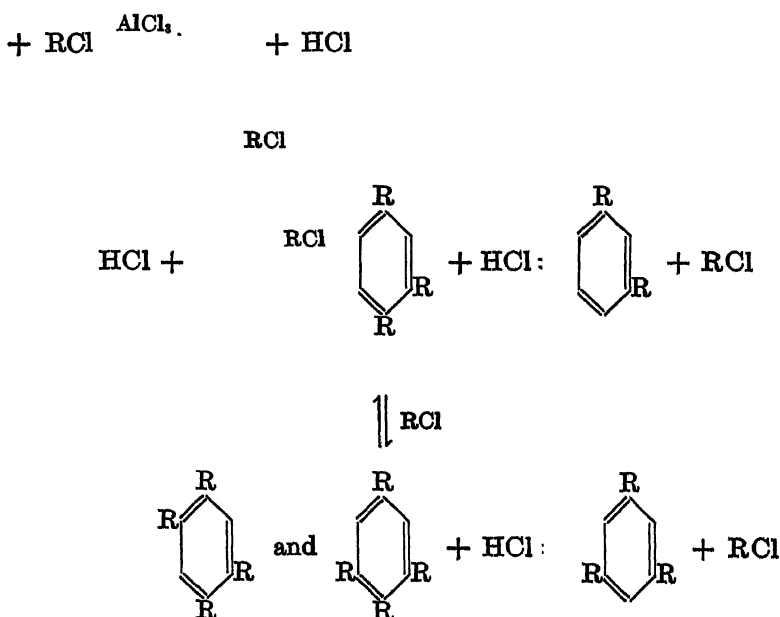
$$k_y = \alpha_1 e^{-e_y e_x / rDRT} \quad (12)$$

In other words, the activation energy for this group of reactions may be determined solely by an expression involving the interaction between the electron density of the reactive group (e_y) and the electron density of the reagent (e_x) at the distance r which separates them in the activated complex.

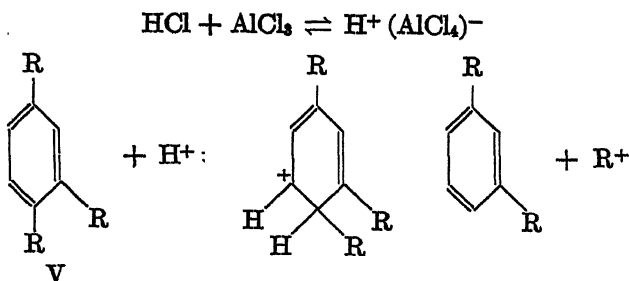
⁸ It must be this *difference* in charge between a carbon atom in benzene itself and in a derivative which was calculated by Eyring and Ri, rather than the absolute value of the charge.

C. Anomalous orientation

One case of orientation which frequently gives results which have been considered anomalous is that of alkylation by the Friedel-Crafts reaction. In many cases of the alkylation of benzene, for instance, using an alkyl halide with aluminum chloride as catalyst, the dialkylated material consists chiefly of the meta isomer, whereas the first alkyl group should orient an incoming group to the ortho and para positions. It has been suggested (45) that this fact is due to the reversibility of the reaction.



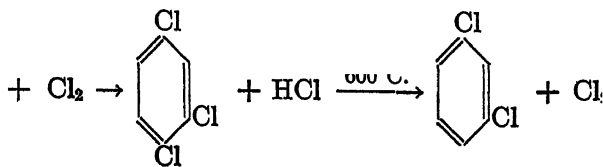
Nightingale and Smith (35), in studying the action of aluminum chloride in rearranging compounds of the type V to the symmetrical isomer, have, in fact, isolated substantial amounts of the *m*-dialkyl compound. This replacement of the alkyl group in the 4-position by hydrogen would take place according to the following steps:



The ease with which the R group will be replaced by hydrogen in this manner will be conditioned by its affinity for the pair of electrons binding it to the carbon atom of the benzene ring. This is indeed true, since a methyl group in this position is much more stable than other alkyl radicals, and *n*-propyl is more so than isopropyl (34).

It is thus apparent that an alkylation performed in such a manner that the reaction is reversible may lead to *m*-dialkyl derivatives, whereas the irreversible alkylations will yield the expected *o*- and *p*-dialkylbenzenes.

This same explanation may be applied to the results of Wibaut and van Loon (59) on the halogenation of benzene at high temperatures. For example, benzene reacts with chlorine above 600°C. to yield *m*-dichlorobenzene rather than the normal *o*- and *p*-isomers. The migration of aromatically bound halogen atoms under certain conditions has already been mentioned; evidently elevated temperatures in the presence of hydrogen chloride may lead to the same result.



IV. SUMMARY

Investigations of the usual substitution reactions in benzenoid compounds involving the replacement of a hydrogen atom, particularly halogenation and the Friedel-Crafts type of reaction, support a mechanism involving attack by an electron-deficient cationoid reagent.

On the basis of such a mechanism, the orientation of an entering group may be effected by the polarization of the double bonds of the benzene ring. This polarization may be accomplished by the electrostatic attraction or repulsion of a substituent group for the electrons of the adjacent double bond in the ring. Since the electrostatic polarizing forces for a large variety of substituents, calculated from dipole moment data and interatomic distances, closely parallel the observed orienting influences of the groups, this force is apparently the principal factor governing orientation.

The polarizing force for a hydrogen atom was estimated to be 0.45 dyne. Groups having a smaller but positive polarizing force are predominantly ortho-para directing, although significant amounts of the *m*-isomeride are usually obtained; groups having a negative (electron-repelling) polarizing force are almost exclusively ortho-para directing. Groups having a polarizing force larger (more electron-attracting) than that of hydrogen (0.45 dyne) but less than 1.0 dyne are principally meta directing, although sig-

nificant amounts of the *o*- and *p*-isomerides are formed; groups having a polarizing force greater than 1.0 dyne are increasingly powerfully meta directing, usually yielding 90 per cent or more of the *m*-isomeride.

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OXIDATION-REDUCTION INDICATORS USED IN VOLUMETRIC ANALYSIS¹

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I. A GENERAL DISCUSSION OF INTERNAL ANALYTICAL OXIDATION-REDUCTION INDICATORS

A. HISTORY OF OXIDATION-REDUCTION INDICATORS

In analytical chemistry there are a great many volumetric analyses that depend upon oxidation-reduction reactions. The usual method of running such an analysis is to obtain the unknown constituent in solution as one ion and to change it to a second ion by adding just enough to a standard solution of an oxidizing agent or a reducing agent. The entire determination depends upon knowing when just enough of the standard solution has been added to carry out the desired reaction. It is possible to determine this equivalence point electrically, but the apparatus is costly and the operation is tedious. Fortunately, some of the standard solutions have one color in the oxidized form and another color in the reduced form, or are colorless in one form. When this is the case and the color change is sharp enough, the standard solution will act as the indicator for the reaction. Unfortunately for this method of analysis, the difference in color between the oxidized and the reduced forms of many of the more suitable oxidizing agents is not great, nor is the color change sharp at the equivalence point. In many analyses other colored ions are present and mask the color change at the end point. Furthermore, the presence of certain reagents added during the process of analysis may interfere with the color change, although they have no color of their own.

In the titration of ferrous ion with potassium dichromate solution the color change at the equivalence point is difficult to detect accurately. In studying this reaction in 1906, Brandt (6) proposed that diphenylcarbazine, $\text{CO}(\text{NHNHC}_6\text{H}_5)_2$, which he called diphenylcarbon-hydrazide, be used as an internal indicator. This compound forms a red color with ferric salts

but is colorless in the presence of an excess of potassium dichromate. Barnely and Wilson (3) in 1913, in attempting to reproduce Brandt's experiments, failed to get concordant results. It was found that the results obtained were more dependent upon the conditions of the analysis than upon the amount of iron in the sample. In a later publication (6) Brandt reported his indicator to be only fairly accurate in macrodeterminations of iron and not applicable at all to microdeterminations.

Modern internal oxidation-reduction indicators really began to become prominent in 1924 with the publication by Knop (34) of a discussion of the use of diphenylamine as an internal indicator in the iron-dichromate titration. Sarver and Kolthoff and their coworkers (48) at the University of Minnesota and Young and Willard and their coworkers (74) at the University of Michigan have applied themselves for some time to a study of the use of diphenylamine, diphenylbenzidine, and their derivatives as oxidation-reduction indicators.

Clark and Cohen and their coworkers (8) in the Hygienic Laboratory of the United States Public Health Service from 1924 through 1928 investigated in great detail the properties of oxidation-reduction indicators that change color at low potentials, mainly the indigosulfonates. These indicators at present are of physiological or biological rather than of analytical importance, and are rarely used in analytical work.

In 1931 Hammett and Walden and their coworkers (22) at Columbia University first began work with the phenanthroline ferrous ion complex of Blau as an indicator in titrations with ceric ion. Knop in Europe has continued his work on oxidation-reduction indicators, and of late has been mainly interested in the triarylmethane dyestuffs.

Today we find that the most popular oxidation-reduction indicators in the order of their preference are as follows: the phenanthroline ferrous ion complex, diphenylaminesulfonic acid, diphenylamine, diphenylbenzidine, Erioglaucin A, Erio Green B, and Setoglaucin O. Without these oxidation-reduction indicators the use of ceric sulfate as a standard oxidizing agent would be greatly restricted. In general, the introduction of internal oxidation-reduction indicators has greatly widened the field of oxidimetry by introducing new standard oxidizing agents and increasing the accuracy of older ones.

B. THEORY OF OXIDATION-REDUCTION INDICATORS

If a solution of a reductant is titrated by means of a suitable oxidant, and, during the course of the titration, the oxidation potential is observed by means of a suitable apparatus, values are obtained which, when plotted, give a curve similar to that in figure 1. If an acid is titrated with a suitable base and the hydrogen-ion concentration is determined by means of a

suitable apparatus, values are obtained which, when plotted, give a curve similar to that in figure 2.

A comparison of these two curves indicates that the oxidation potential bears almost the same relation to oxidation-reduction titrations that the pH bears to neutralizations. This conclusion has been established by Kolthoff and other workers (28, 29, 30, 31, 32, 33). As an acid-base

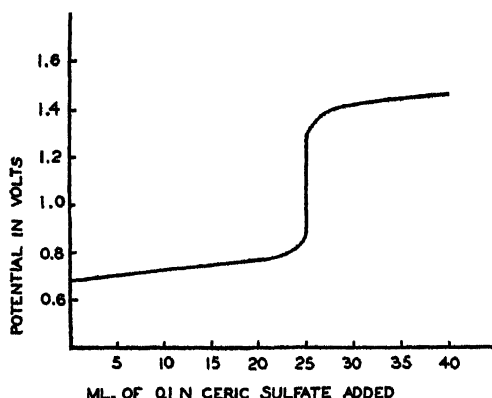


Fig. 1. Titration of ferrous sulfate with standard ceric sulfate

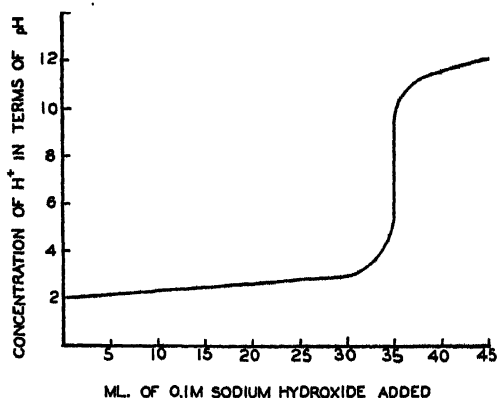


Fig. 2. Titration of sulfuric acid with standard sodium hydroxide

indicator is a compound that produces a marked color change with a certain change of pH, so an oxidation-reduction indicator is a compound that produces a marked change of color with a certain change of oxidation potential. Furthermore, as the ability to change color with change of pH is not the only criterion of the suitability of a compound as an acid-base indicator in a neutralization, so the ability of a compound to change color

with a change of oxidation potential is not the only criterion of its suitability as an indicator in an oxidation-reduction titration.

From the oxidation potential of the constituents titrated and from that of the reducing agent, it is possible to calculate the potential at the equivalence point. Theoretically, therefore, it is possible to calculate the range of oxidation potential in which that of an indicator must be located to be suitable. In the selection of the proper indicator upon the basis of such theoretical considerations, it should be realized that in most cases the oxidation potential at the equivalence point depends upon the hydrogen-ion concentration and the concentration of the other ions present in the solution. Furthermore, it is frequently found that these anions or cations affect the oxidation potential of the constituent titrated, or that of the reagent, or both, usually as a result of complex ion formation. Thus the suitable range in which the color change of the indicator must occur becomes dependent upon the nature of the solution being titrated.

The range of oxidation potential in which the color change of the indicator actually lies also depends upon the solvent medium. As a general rule, oxidation-reduction indicators in their oxidized and reduced forms are donors (acids) or acceptors (bases) of protons, respectively. Their oxidation potentials, therefore, are more or less complicated functions of the hydrogen-ion concentration of the solution and, in addition, depend upon the ionic strength of the medium. That is, the oxidation potential at which the indicator shows a color change varies with different values of the pH and also when different kinds of ions are present. That this is a complicated function is shown by the following equation, which was derived by Cohen and Clark (8, 9) for the variation of the oxidation potential of the simple indophenols at 30°C.:

$$E_h = E_0 - 0.03006 \log \frac{C_r}{C_o} - 0.03006 \log [K_r K_2 (H^+) + K_r (H^+)^2 + (H^+)^3] - 0.03006 \log [K_o + (H^+)]$$

where E_h = the oxidation potential with respect to the normal hydrogen electrode,

E_0 = the oxidation potential when $C_r/C_o = 1$ and the pH = 0,

C_r = the concentration of the total reductant,

C_o = the concentration of the total oxidant,

K_o = the dissociation constant of the oxidant,

K_r = the acid dissociation constant of the hydrogen in the reductant to which K_o applies in the oxidant,

K_2 = the acid dissociation constant of the phenolic group, created by the reduction, and

H^+ = the concentration of the hydrogen ion.

As this equation is given for a specific temperature, we know that the oxidation potential of the indicator will vary with the temperature.

The considerations in the two preceding paragraphs are not so very important in cases where the jump in potential near the equivalence point is very great, but they become of primary importance where the useful range of oxidation potential becomes small. Because of these difficulties, most of the investigations of analytical oxidation-reduction indicators have been confined to systems in which the difference in potential just before and just after the equivalence point is fairly great, such as in titrations of ferrous ion with ceric sulfate or potassium dichromate.

The change in the structure or the composition of the indicator that causes the color change is not known in all cases. Blau and later workers have prepared the ferrous phenanthroline complex and found it to be red, while the ferric phenanthroline complex was found to be blue. It is generally accepted that the color change in this indicator is caused by the change from the ferrous to the ferric complex or *vice versa*, and that the ferrous ion held in the complex is not oxidized until an oxidation potential of 1.14 volts with reference to the normal hydrogen electrode is reached.

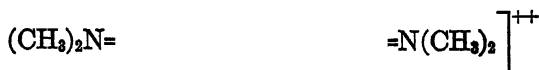
Kolthoff and his coworkers (28, 29, 30, 31, 32, 33) have investigated thoroughly the color change that takes place in diphenylamine. Their experiments show that the first excess of oxidant changes diphenylamine to diphenylbenzidine. Both of these compounds have been isolated and their composition and structure determined; both are colorless, and the reaction changing the former to the latter is irreversible. This means that this reaction must take place at a lower potential than the equivalence potential or within the useful range. The next portion of oxidant produces a violet coloration in dilute solutions. This violet compound is called diphenylbenzidine violet and is shown, by its absorption spectrum, to be a quinoid salt. Efforts to isolate diphenylbenzidine violet failed, but as a result of spectrographic and other studies the following structure was assigned to it:

Diphenylbenzidine violet

If the portion of oxidant used to produce the violet color is added slowly to a concentrated solution of the indicator, a green compound is produced which disappears on the addition of more oxidant, giving the violet compound. The investigators called this green compound diphenylbenzidine green. It is assumed to be a molecular compound, called a meriquinoid, the product of the reaction between the oxidized diphenylbenzidine (diphenylbenzidine violet) and the unoxidized portion of diphenylbenzidine.

In ordinary titrations diphenylbenzidine green is not produced, because of the small quantity of indicator used and its dilution. However, the oxidation potential of the color change is thought to be the equilibrium potential for the reaction diphenylbenzidine green \rightleftharpoons diphenylbenzidine violet.

Kehrman (26) and his associates established compounds I and II, respectively, as the oxidation products of the two arylmethane dyes Malachite Green and Brilliant Green.



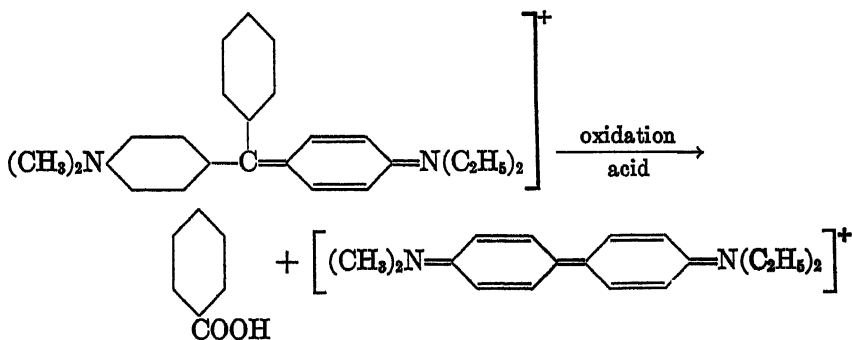
Oxidation product of Malachite Green



II

Oxidation product of Brilliant Green

As can be seen by an examination of the formulas, the compounds are benzidine derivatives and could theoretically be prepared from benzidine. Knop, in attempting to explain the cause of the difference in color of the oxidized and reduced forms of some twenty-five triarylmethane dyes on the basis of this formula, regarded the following reaction as typical:



Absorption spectra studies and comparisons with derivatives of benzidine proved this to be the case in only a few instances, and never when the oxidation product was rose colored. The rose-colored product could not be identified nor is its structure known.

Knop concluded that, when one of the triarylmethane dyes was used as an indicator, during the oxidation there was formed, by an irreversible reaction, a new compound that became the indicator for the reaction. On reduction, this new compound produced a product which was similar

in color to the original dye but was not the original dye. Knop substantiated this contention with experimental evidence, although he was unable to identify definitely the oxidation products. In general, it is thought that the color change in the derivatives and substitution products of diphenylamine is caused by a change similar to that in diphenylamine, and that the color change in the *p*-nitrophenanthroline ferrous complex is similar to that of the phenanthroline ferrous complex. The remaining oxidation-reduction indicators have not been fully studied, and the exact change that causes the color shift has not been determined for every indicator.

Just as with acid-base indicators the color change is sometimes gradual over a range of pH, with a sharp change within certain limits, so with oxidation-reduction indicators there is often more than one color change, but usually one change is sharper between certain limits than any of the others. In the case of oxidation-reduction indicators a single value of the oxidation potential is usually given. This is the potential at which the indicator shows its greatest color change, and it is given with reference to the normal hydrogen electrode, unless otherwise specified. Usually, if the pH is not given, the color change takes place at the given potential in solutions which are between 1 and 2 normal with respect to hydrogen ion.

A system of indicating the range of oxidation-reduction indicators other than that of the oxidation potential which is sometimes, although infrequently, used is that of sensitivity. When it is said that diphenylamine has a sensitivity of 4×10^{-6} to potassium permanganate in 1 *N* sulfuric acid, this means that as soon as the 1 *N* sulfuric acid containing the indicator becomes 4×10^{-6} *N* with respect to potassium permanganate the indicator becomes violet colored. As all oxidizing agents of the same normality do not have the same oxidizing power, this system does not possess the advantage of suggesting the suitability of an indicator for use with different oxidants and reductants that the oxidation potential method does.

C. CHARACTERISTICS OF OXIDATION-REDUCTION INDICATORS

An oxidation-reduction indicator is defined as a substance the oxidized form of which has a color different from that of the reduced form. The character of this color change (when it will take place), the solubility, the reactivity, and the stability of the substance are the main characteristics that must be considered in determining its suitability as a general oxidation-reduction indicator.

(a) The oxidation potential at which the color change takes place is of prime importance. In most of the titrations to which these indicators have been applied this potential must be fairly high,—between 0.7 and 1.3 volts. It is not correct to say that the potential at the transition point (from the

reduced to the oxidized form) must be in the neighborhood of 1.0 volt, because that will depend upon the titrating reagent and the constituent being determined. There is no reason to believe that in the future oxidation-reduction indicators may not be applied to determinations that have a relatively low potential at the equivalence point.

(b) It is essential that the color change at the transition point be sharp. Not only should the difference in color of the oxidized and reduced forms be marked, but the change from one form to the other should be very rapid. Smith (53) gives as the most desirable change one from red to violet or blue. Not all analysts agree with this statement. Some prefer a single-color indicator, believing that a change is best also if this color is so marked that it stands out above colors that might be imparted to the unknown solution by frequently occurring anions or cations.

(c) The end-point color should be fairly permanent. The compound should therefore be stable with respect to both oxidant and reductant. The end color should last for at least 2 min., and it is better if it lasts much longer. Unfortunately, many of the indicators produce colors that last only a few seconds.

(d) In most titrations it is necessary for the indicator to be resistant to the action of the common mineral acids. It is possible that future applications may be made in basic or weakly acidic solutions, but, at present, titrations to which the indicators have been applied are carried out in fairly strong acid solutions (at least 1 to 2 *N* with respect to hydrogen ion). It is well, also, if the indicator is resistant to temperatures almost as high as 100°C.

(e) The indicator should not react with the commonly occurring anions and cations. Such action would interfere with the end-point color by forming a new compound or complex that might have different properties.

(f) It is desirable that the indicator reaction be reversible, since this will allow overstepped end points to be back-titrated. However, although certain of the oxidation-reduction indicator reactions are not reversible, they are still used, mainly those used with potassium bromate, most of which are destroyed by the bromine released at the end point. Unfortunately, with an irreversible indicator the end point may be approached from one side only. If the indicator is acceptable in other respects, an irreversible indicator reaction should not cause it to be entirely discarded.

(g) It is preferable that the indicator be water soluble. Limited solubility is just as acceptable as decided solubility, for indicator solutions are seldom stronger than 2 per cent. If the indicator is not soluble in water, the next best property is for it to be soluble in the common acids like sulfuric, hydrochloric, or acetic acid. The indicator is somewhat less desirable if it is soluble in organic solvents only.

(h) The indicator is more acceptable if its solutions are stable for some

length of time, although this is not absolutely essential, as new solutions could be made for each set of determinations. However, if the solutions are stable, the indicator lends itself more readily to routine work.

(i) The indicator will be more acceptable if only small quantities are needed for each determination. If a large quantity is needed, this will require a larger quantity of oxidant or reductant to cause the color change and will increase the value of the blank.

(j) It is preferable that the indicator be easily prepared or readily obtainable. Of course, if a compound that proves itself to be a good indicator is difficult to prepare, chemical supply houses will soon make it available.

II. SPECIFIC INTERNAL ANALYTICAL OXIDATION-REDUCTION INDICATORS

A. INDICATORS OF THE DIPHENYLAMINE SERIES

These indicators, at least theoretically, may be formed from diphenylamine by the substitution of different groups for one or more of the hydrogens of the benzene rings. They all contain the structure



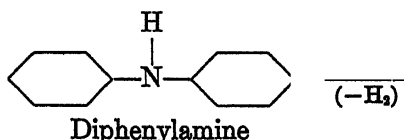
where atoms or groups other than hydrogen may be present on the rings.

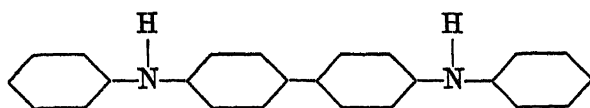
This is the oldest group of oxidation-reduction indicators and one in which investigations are still being carried on. As a general rule, the color changes are from colorless to some shade of blue or pink. Also, as a general rule the introduction of carboxyl or sulfonic acid groups increases the oxidation potential necessary to cause a color change.

In general, for the members of this group as well as for the other indicators, the following five characteristics and conditions will be given in order: (a) structure of the indicator; (b) preparation of the indicator; (c) preparation of the indicator for use; (d) use of the indicator; (e) limitations of the indicator.

1. *Diphenylamine* (1, 15, 18, 19, 20, 21, 24, 28, 32, 33, 34, 41, 46, 48, 50, 52, 66, 68, 74, 75)

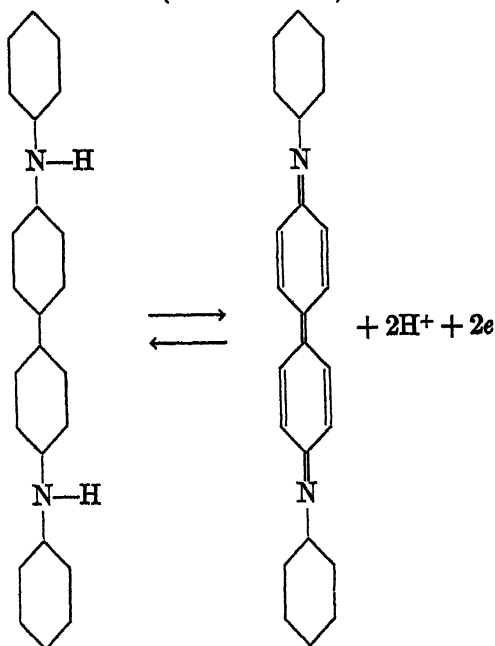
(a) *Structure:*



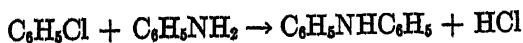


Diphenylbenzidine

(colorless forms)

Diphenylbenzidine
violet

(b) *Preparation of the indicator:* Diphenylamine can be prepared according to the reaction:



Sodium hydroxide is used to bind the hydrogen chloride formed. A mixture of 37 g. of aniline, 44 g. of chlorobenzene, and 40 g. of soda lime is heated for 34 hr. or longer in an autoclave at a temperature of 360–370°C. The mass is dissolved in 800 cc. of water and neutralized with hydrochloric acid. The unchanged aniline dissolves, and the diphenylamine separates as an oil which solidifies overnight and can be filtered off. The product may be further purified by recrystallization from ether.

The indicator is sold in bulk form by most chemical supply houses, by

the Eastman Kodak Company, and by the National Aniline and Chemical Company.

(c) *Preparation of the indicator for use:* Method A—1.69 g. of diphenylamine is melted and is then dissolved, by shaking, in 1 liter of concentrated sulfuric acid. Five or six drops of this solution are used with 0.1 *N* reagents and 0.05–0.02 cc. with weaker reagents. A 0.1 per cent solution in concentrated sulfuric acid is preferred by some analysts.

Method B (preparation of the oxidized form)—According to Young and Willard (74, 75), the correction due to the indicator can be greatly reduced by using the indicator in the oxidized form, which is prepared in the following manner: 0.1 g. of the indicator is dissolved in 10 cc. of sulfuric acid (sp. gr. 1.83) and the solution is diluted with 90 cc. of glacial acetic acid. To about 10 cc. of the above solution in a small beaker is added 5 cc. of phosphoric acid (sp. gr. 1.37) and three or four drops of 0.1 *N* potassium dichromate, followed by very dilute ferrous sulfate solution until a single drop causes the solution to become colorless. Small portions of the indicator so prepared are used.

(d) *Use of the indicator:* The oxidation potential of the indicator at the color change is 0.75 volt and is nearly independent of the hydrogen-ion concentration.

The indicator is one of the best for the determination of iron by titration with potassium dichromate. Knop (34, 35) states that Penny's (41) method for the determination of iron, using potassium dichromate as the standard oxidizing agent with diphenylamine as an indicator, is more accurate than the usual Rheinhardt-Zimmerman method, using potassium permanganate without an indicator. Diphenylamine can be used as an indicator in the determination of cobalt by titration with standard potassium dichromate. It is satisfactory as an indicator in the titration of uranium with potassium dichromate. When hydrofluoric acid is used to suppress the ferrous-ferric potential, accurate results can be obtained on iron in a silicate rock by using the indicator and titrating with standard potassium dichromate. Knop (34, 35) thoroughly investigated the use of the indicator in ferrous-dichromate titrations and found it well suited for use in either macrodeterminations or microdeterminations of iron in ore or in steel. He found that the presence of zinc, nickel, aluminum, cobalt, or chromium has no effect on the determination of iron when this indicator is used. The same investigator also found that dichromate could be employed as the titrating agent when the indicator was used in determining ferrous iron in mixed ferric-ferrous solutions, and that the presence of the ferric ion did not interfere in quantities less than 1 g. in 150–200 cc. of solution.

One of the best methods of determining chromium and vanadium in alloy steels by titrating with ferrous sulfate solution makes use of diphenylamine as the indicator. Ackermann (1) reports using the indicator satisfactorily in the direct titration of used chromeliquor in the tanning of leather. Knop (34, 35) applied the indicator to the determination of chromium in steel. He found that accurate results could be obtained by dissolution of the sample in 1:1 sulfuric acid, oxidation with ammonium persulfate, removal of the manganese as the oxyhydroxide, reduction of the chromic acid so formed with standard ferrous sulfate solution, and back-titration with standard potassium dichromate. Furman (19) found the indicator useful in the determination of vanadium in ores or steels even in the presence of ferric iron, quinquivalent arsenic, and hexavalent uranium. He employed various methods and titrated with standard ferrous sulfate after oxidizing with potassium permanganate.

Diphenylamine can be used in the titration of hydroquinone with standard ceric sulfate solution, and it can also be applied to the titration of ferrous salt solutions with ceric sulfate. Young and Willard (74, 75) found that the indicator accurately determined the equivalence point in the ferrous-ceric titration even in the presence of arsenious acid and that results so obtained were fully as accurate as potentiometric titrations. They used a buffer solution of sodium acetate solution for all solutions containing free hydrochloric or sulfuric acid. The same investigators substantiated the previous work of Knop on the same indicator.

Diphenylamine is a preferred indicator in the titration of potassium ferrocyanide with standard zinc sulfate.

If diphenylamine is added in the permanganate titration of iron, the end point is sharper and more permanent than when no indicator is used; furthermore, the presence of hydrochloric acid, stannic chloride, or mercurous chloride does not interfere with the end color, although a mixture of 15 cc. each of concentrated sulfuric and phosphoric acids should be used in each 100 cc. of solution. The indicator has been found especially useful in the determination of iron in solutions containing chromium, cobalt, or nickel with standard permanganate; where ordinarily the color of the solution would interfere with the end point, the intense blue color of the diphenylamine prevails.

It has been found that, in general, organic materials do not interfere with titration end points where diphenylamine is used as the indicator.

(e) *Limitations of the indicator:* The oxidation-reduction potential at the color change is so low that in iron determinations the ferrous-ferric potential must be lowered by the addition of fluoride or phosphate to form

a complex. The color of the indicator fades on standing, and the indicator is destroyed by an appreciable excess of oxidant. When the oxidized form of the indicator is used, the indicator correction is negligible, but when the unoxidized form of the indicator is used, it requires a correction in the neighborhood of 0.1 cc. of 0.1 *N* oxidant for each 0.1 cc. of the indicator solution used. Furthermore, it requires different amounts of different oxidants of the same normality to cause the same amount of the indicator to change color. Also, if the titration is not carried out immediately after adding the indicator (within 15 min.), the results will not be accurate. Moreover, there is a reaction between the oxidized and unoxidized forms of the indicator that affects the color at the end point in certain determinations.

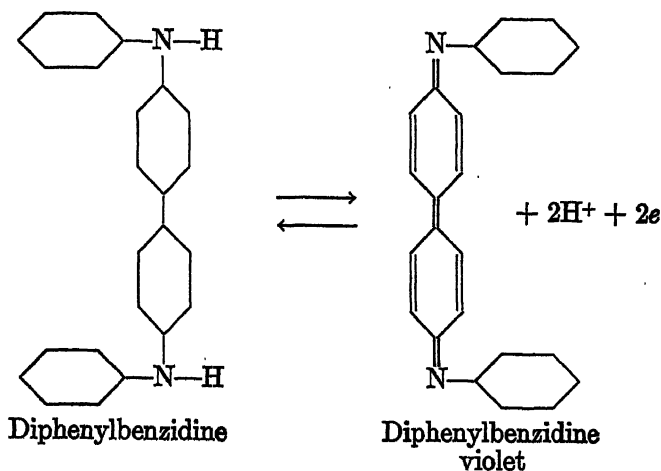
The end-point color is not good in the dichromate-iron titration unless the ferric ion is suppressed with phosphoric acid or hydrofluoric acid. The iron-dichromate reaction, using the indicator to denote the end point, is not quantitative in the presence of copper or trivalent arsenic. Copper produces low results unless it is present in quantities less than 1 mg. in 150-200 cc. of the solution being titrated; trivalent arsenic produces high results. Knop (34, 35) found that, in the iron determination with standard dichromate by reduction with stannous chloride and destruction of the excess with mercuric chloride, a small error, equal to about 0.1 per cent of the result, is introduced. He found also that small quantities of platinum affected the results slightly, so care must be exercised where platinum vessels are used in taking the sample into solution. Some investigators find that certain persons have difficulty obtaining accurate results in the potassium dichromate-ferrous ion titrations, as the end point is not as sharp as some writers would lead one to believe.

In the hydroquinone-ceric ion titration there is an appreciable blank correction, equivalent to about 0.05 cc. of 0.1 *N* ceric sulfate for each 0.05 cc. of 1 per cent indicator solution used. The indicator is unreliable in the determination of cerium with ferrous sulfate. To secure accuracy in the determination of ferrous iron by ceric ion a suppressing agent must be used, and even then the blank correction is appreciable.

Scott (52) observed that when diphenylamine is used in the permanganate-iron titration to sharpen the end point, more than 0.4 cc. of the indicator affects the results. Sarver (46) found that end points with diphenylamine are often passed without the formation of color. Watson proved that mercuric chloride has a powerful inhibiting effect upon color formation with the indicator.

Diphenylamine cannot be used to detect the end point in determining iron when tungstate ion is present.

2. Diphenylbenzidine (32, 35, 47, 48, 71, 75)

(a) *Structure:*

(b) *Preparation of the indicator:* To a mixture of 1 liter of concentrated sulfuric acid, 2 liters of glacial acetic acid, and 12 liters of water is added slowly, and with constant stirring, a solution of 50 g. of diphenylamine in 500 cc. of acetic acid. If diphenylamine separates, it may be redissolved by heating not higher than 50°C. To the resulting solution at 15–25°C. is added slowly and with vigorous stirring a solution of 22 g. of potassium dichromate in 500 cc. of water; after 15 min. the excess dichromate is reduced with a solution of 10 g. of sodium bisulfite in water. The colloidal precipitate is collected on a filter with the aid of a filter medium. The greenish black precipitate is placed, while still wet, in a warm concentrated solution of sodium bisulfate; when it turns light brown it is filtered off, washed with hot water, thoroughly dried, pulverized, and extracted with the same solvent. The pure diphenylbenzidine separates on cooling. It is filtered off, washed with alcohol until the washings fail to yield a white cloud upon dilution with water, and dried at 110°C. The yield is 60 to 65 per cent.

(c) *Preparation of the indicator for use:* Method A—A 0.1 per cent solution in concentrated sulfuric acid is prepared. One drop of this solution is mixed with 10 cc. of a mixture of equal parts of glacial acetic acid and sirupy phosphoric acid. The entire amount is used in an iron determination with 0.01 *N* or stronger reagents for good sharp end points.

Method B—A 0.005 *M* solution is prepared by dissolving 1.68 g. of diphenylbenzidine in 1 liter of 9 parts of glacial acetic acid and 1 part of concentrated sulfuric acid. Five or six drops are used when titrating with

0.1 *N* oxidant in 100–200 cc. of volume. When 0.01 *N* or 0.001 *N* solutions are to be titrated, 0.02–0.05 cc. of the solution is used in 10–50 cc. of volume.

Method C (preparation of the partially oxidized form)—0.1 g. of diphenylbenzidine is dissolved in 10 cc. of sulfuric acid (sp. gr. 1.83), and the solution is diluted with 90 cc. of glacial acetic acid. To about 10 cc. of the above solution in a small beaker are added 5 cc. of phosphoric acid (sp. gr. 1.37), three or four drops of 0.1 *N* potassium dichromate, and then very dilute ferrous sulfate solution until a single drop causes the solution to become colorless. Small portions of this solution are used.

(d) *Use of the indicator*: In general, the indicator may be used in the same determinations as diphenylamine. According to the theory of oxidation–reduction indicators, diphenylamine is oxidized by the first excess of oxidant to diphenylbenzidine; therefore the oxidation potential at the end point will be the same for both indicators,—namely, 0.73 volt.

As would be expected from the above, the indicator correction with diphenylbenzidine is less than that with diphenylamine. It amounts to about 0.1 cc. of 0.01 *N* oxidant for each 0.1 cc. of diphenylbenzidine prepared by method B, and about twice this quantity when diphenylamine is used. In titrating dichromate or vanadate with ferrous sulfate the correction is about the same. This is not true with diphenylamine.

Diphenylbenzidine can be used in ceric titrations of iron, even in the presence of arsenious acid; however, it is less satisfactory than diphenylamine. Diphenylbenzidine is also applicable to the determination of vanadium by titration with ceric sulfate.

(e) *Limitations of the indicator*: Diphenylbenzidine is subject to essentially the same limitations as diphenylamine, since it is the partially oxidized form of diphenylamine. Young and Willard (74, 75) report less success with the partially oxidized form of diphenylbenzidine than with the partially oxidized forms of either diphenylamine or diphenylamine-sulfonic acid. This is to be expected from the theory, as the next step in the oxidation of diphenylbenzidine would produce the color change; this is not true with diphenylamine or diphenylaminesulfonic acid. The same investigators report that when diphenylbenzidine is used in the ceric titration of iron in the presence of arsenious acid a heavy white precipitate is formed, which is not formed with diphenylamine in the same determination.

3. Diphenylaminesulfonic acid (33, 48, 74)

(a) *Structure*: The position of the sulfonate radical in this compound has not been established. The formula usually given is $\text{HSO}_3\text{C}_6\text{H}_4\text{NHC}_6\text{H}_5$.

(b) *Preparation of the indicator*: 170 g. of diphenylamine is mixed with 95 ml. of acetic anhydride and 10 ml. of concentrated sulfuric acid, and the

mixture is refluxed for 1 hr. The excess acetic acid is removed by washing with cold water. The acetyldiphenylamine is recrystallized from ether and dried at 100°C. Two hundred grams of 20 per cent fuming sulfuric acid is cooled to 5°C. and to this is added 100 g. of pulverized acetyldiphenylamine in small portions with constant stirring. The mixture is then warmed for 2 hr. on a water bath at 45°C. with constant stirring. It is poured into 250 cc. of water and boiled for 3 hr. to hydrolyze the acetyl compound. The mixture is then poured into a large volume of water, and the unchanged diphenylamine is filtered off. The filtrate is neutralized with barium carbonate, and the barium sulfate is filtered off. The white barium diphenylaminesulfonate and the barium acetate are separated by fractional crystallization.

(b) *Preparation of the indicator for use:* Method A—A 0.005 *M* solution of the barium salt of the acid is prepared by dissolving 3.17 g. of the anhydrous salt in water.

Method B—A 0.01 *M* solution of sodium diphenylaminesulfonate is prepared by dissolving 3.2 g. of the barium salt in water, adding sodium sulfate in excess, and decanting from the barium sulfate. One hundred cubic centimeters of this solution and 25 cc. of concentrated sulfuric acid are placed in a volumetric flask and diluted to 900 cc. To this is added 25 cc. of 0.1 *N* potassium dichromate, slowly and with frequent stirring, and then 0.1 *N* ferrous sulfate with repeated shaking until one drop causes a visible color change from bluish green to a clear deep green in the liquid when viewed through the neck of the flask; this requires about 6.5 cc. of ferrous sulfate solution. This solution is used as the indicator, but it settles on standing and requires frequent shaking.

Method C—A mixture of 100 cc. of the 0.01 *M* sodium diphenylaminesulfonate and 5 cc. of concentrated sulfuric acid is diluted to about 300 cc. To this is added about 25 cc. of 0.1 *M* potassium dichromate, 8 cc. of 0.1 *N* ferrous sulfate, and 5 cc. of concentrated sulfuric acid. The mixture is allowed to stand 3 or 4 days, until the supernatant liquid gives no color when added to 100 cc. of water containing 2 cc. of 0.1 *N* potassium dichromate and 5 cc. of concentrated sulfuric acid. The supernatant liquid is siphoned off slowly, care being taken not to disturb the green precipitate, which is the indicator. After the addition of 300 cc. of water and 15 cc. of concentrated sulfuric acid, the material is allowed to settle again. The siphoning and washing are repeated. More rapid washing can be obtained by means of the centrifuge. The precipitate is then shaken up with 100 cc. of water, in which, in the absence of an electrolyte, it remains in colloidal suspension. For most titrations 0.5 cc. of this solution is sufficient.

Method D—A stock solution is prepared containing 0.2 to 0.5 per cent of either sodium or barium diphenylaminesulfonate in water.

(c) *Use of the indicator:* Diphenylaminesulfonic acid is one of the best of all indicators in the titration of ferrous ion with potassium dichromate, the color change being so brilliant and so sharp that it can be carried out even in brilliantly colored solutions. The indicator may be used in the presence of tungstate ion; this is not true of diphenylamine or diphenylbenzidine. Sarver and Kolthoff (48) also used the indicator in permanganate or vanadate titrations in acid solutions. It can be used also as the reverse indicator, that is, in titrating vanadate or dichromate with ferrous ion. The indicator can be used internally in the titration of zinc with potassium ferrocyanide or the reverse. It can be used in almost every dichromate titration and in many ceric titrations, especially in the ceric titration of iron.

(d) *Limitations of the indicator:* None of the diphenylamine derivatives is very resistant to excess oxidant, as previously stated. However, diphenylaminesulfonic acid resists it better than the other members of the series. The color change is inhibited by mercury salts. The color change in dichromate titrations other than that of iron is somewhat slow; apparently it is catalyzed by ferrous ion. The indicator cannot be used in all ceric titrations. When ferrous ion is titrated with either ceric sulfate or potassium dichromate, the ferrous-ferric potential must be lowered by adding about 10 cc. of 25 per cent phosphoric acid for each 50 cc. of titrating volume.

The indicator correction with this indicator is appreciable. The correction amounts to about 0.1 cc. of 0.01 *N* oxidant or its equivalent for each 0.25 cc. used. In back-titrations with ferrous ion, the correction is somewhat greater. Practice dictates that the indicator correction be determined by running a blank along with the determination.

4. Monosulfonate of diphenylamine (11)

(a) *Structure:* The exact structure of this compound is not known. The formula given is $C_6H_5NHC_6H_4SO_3Na$. It is probable that the compound is the sodium salt of an isomer of the diphenylaminesulfonic acid of Kolthoff and Sarver (33).

(b) *Preparation of the indicator:* A mixture of 21 g. of di-*n*-butyl sulfate and 16.9 g. of diphenylamine is heated on a boiling water bath for 1 hr. and then on a metal bath at 130–140°C. for 1 hr. A solution of sodium ethylate (4.8 g. of sodium and 200 cc. of ethanol) is added, and the mixture is refluxed until the tarry reaction mass becomes white. The alcohol is distilled off, the residue is taken up in water, the mixture is filtered, and the filtrate is thoroughly extracted with ether to remove residual diphenylamine. The ether is removed by warming and aeration. Concentrated sodium hydroxide is added cautiously until a faint turbidity appears. The

desired sodium salt precipitates slowly. After 24 hr. the suspension is filtered off, and the salt is sucked as dry as possible. The salt is redissolved in warm ethyl alcohol and precipitated with an excess of ether. Purification is completed by dissolving again in warm alcohol, passing in carbon dioxide until no more sodium carbonate separates, filtering, and concentrating the filtrate to incipient crystallization on the water bath.

(c) *Preparation of the indicator for use:* A 0.1 per cent solution of the sodium diphenylaminesulfonate in water is prepared. Four or five drops of this solution are used in 400 cc. of titrating solution.

(d) *Use of the indicator:* The indicator has been applied to the iron-dichromate titration. The indicator correction is negligible. The color change is from colorless in the reduced form to deep purple in the oxidized form, and the end color lasts about 15 min. Mercury salts do not inhibit the color change. Analyses of iron and chromium ores show it to be reliable.

(e) *Limitations of the indicator:* In the one determination to which the indicator has been applied, the usual sulfuric-phosphoric acid mixture must be used,—25 cc. of the mixed acids in each 400 cc. of titrating solution. This indicator has possibilities, but has not been thoroughly investigated or applied to enough determinations.

5. *Product of the action of ethyl sulfate on acetyldiphenylamine (25, 57)*

(a) *Structure:* The structure is undetermined. The substance is probably the sodium salt of a monosulfonated diphenylamine which, in turn, is probably an isomer of diphenylaminesulfonic acid.

(b) *Preparation of the indicator:* A mixture of 50 g. of acetyldiphenylamine, prepared by the method of Kaufmann (25), and 40 g. of ethyl sulfate is heated on a water bath for 5 hr. The reaction mixture is refluxed with 7 g. of sodium in 200 cc. of ethanol until the original dark-colored mass is converted into a white mass. The alcohol is removed by distillation, and the solid residue is dissolved in water. The solution is extracted thoroughly with ether to remove any diphenylamine. The ether is removed from the water layer by aeration, and the solution is treated with concentrated sodium hydroxide until a permanent turbidity appears. A voluminous precipitate appears within 12 to 24 hr. and may be recrystallized from water.

(c) *Preparation of the indicator for use:* A 2 per cent aqueous solution is used, and three drops per 250 cc. of titrating volume are sufficient for a determination.

(d) *Use of the indicator:* The discoverers of the indicator claim that it can be used wherever diphenylaminesulfonic acid can be used. They state that it is very soluble in water, is unaffected by mercury salts, and is stable

toward excess oxidant, and that the indicator blank is small. The color change, which is from colorless to very red, is very sharp, permanent, and very mobile.

(e) *Limitations of the indicator*: It has been applied only to the titration of ferrous solutions with standard dichromate solution and to the titration of dichromate solutions with standard ferrous sulfate solution.

6. Diphenylbenzidinesulfonic acid (49)

(a) *Structure*: Analysis indicates that when the indicator is prepared as directed, ten sulfonate groups are introduced in most cases. If 25 per cent fuming sulfuric acid is used instead of the specified 60 per cent acid, only four sulfonate groups are introduced. The positions of the groups are unknown and would be difficult to determine.

(b) *Preparation of the indicator*: 1 g. of dry powdered diphenylbenzidine is added, little by little over a period of about 2 min., to 10 cc. of 60 per cent fuming sulfuric acid in a shallow dish. The mixture heats up but should not be allowed to become warmer than 40°C. As complete solution as possible is obtained by stirring and mashing the lumps with a rod; then, after not more than 3 min., the mixture is poured on ice. It is not desirable to work with larger quantities, because the reaction is difficult to control. However, as many portions as desired can be combined at this point. For each gram of diphenylbenzidine used, the solution is diluted to a volume of 200 cc.

The solution is heated to boiling and the green color is reduced with a stream of sulfur dioxide. The excess sulfur dioxide is removed by boiling, and the solution is filtered to remove any tarry matter. The filtrate is carefully neutralized with sodium carbonate, more water being added if any precipitation occurs. Four volumes of ethyl alcohol are added to precipitate the sodium sulfate. After filtration, the filtrate is retained. The residue is redissolved in water and reprecipitated with alcohol as often as necessary to remove the yellow color from the precipitate. The separate filtrates are combined, the volume being about 5 liters for each gram of diphenylbenzidine used. The alcohol is boiled off rapidly; the evaporation is completed on the steam bath, long exposure or overheating of the residue being avoided. The sodium diphenylbenzidinesulfonate should be a light yellow in color. The yield is 4 g. (approximately) for each gram of diphenylbenzidine used.

(c) *Preparation of the indicator for use*: A 0.1 per cent solution of the sodium diphenylbenzidinesulfonate in water is recommended and can be kept for several months. One drop or less is used with 0.01 or 0.001 *N* oxidizing or reducing solutions and 10 drops with 0.1 *N* solutions.

(d) *Use of the indicator*: The article (49) gives no specific determina-

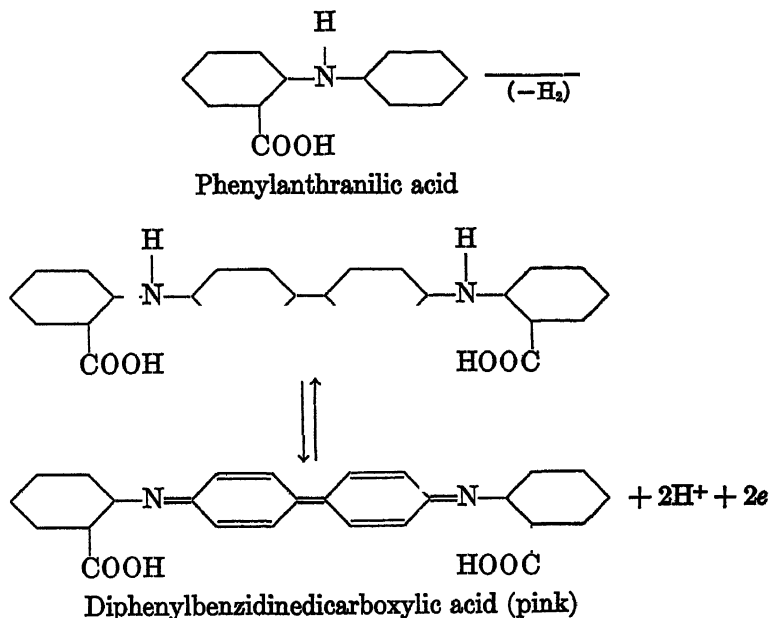
tions in which the indicator has been used, but indicates that it can be used wherever diphenylaminesulfonic acid can be used. Apparently the potential is slightly above that of diphenylaminesulfonic acid at the color change, as substituted sulfonic acid groups in general raise the oxidation potential at the color change. This would make the indicator applicable to titrations of potassium dichromate, potassium permanganate, and ceric sulfate. The color change is pale yellow to deep yellow to violet. The reaction is reversible by reduction.

The indicator is unchanged by contact for 10 min. with an appreciable excess of potassium dichromate. The green half-oxidized form is stable, but the reddish violet form fades after a long time.

(e) *Limitations of the indicator:* The indicator has not been thoroughly enough reported to determine all its limitations. The investigators state that the indicator correction is negligible except in microanalysis. They report, however, that in potassium dichromate determinations back-titration with ferrous sulfate is more reliable than direct titrations. The indicator probably has the limitations of diphenylbenzidine and diphenylamine, modified by greater resistance to excess oxidant and greater resistance to acids, along with increased solubility in water.

7. Phenylanthranilic acid (*o*-diphenylaminecarboxylic acid) (10, 48, 58)

(a) Structure:



There is no mention in the literature of specific experiments to establish the above mechanism as that of the color change, but it is established on the basis of Sarver and Kolthoff's theory of indicators which are derivatives of diphenylamine.

(b) *Preparation of the indicator:* A mixture of 10 g. of *o*-chlorobenzoic acid, 10 g. of potassium carbonate, 40 cc. of aniline, and 0.1 g. of precipitated copper is boiled, in a round-bottom flask fitted with a short upright air condenser, at such a rate that a little water may escape from the top of the condenser. The mixture gradually darkens, and the reaction is complete in about 3 hr. The unchanged aniline is removed by steam distillation and the residue is filtered hot. The latter is boiled with animal charcoal and the mixture is filtered. Upon the addition of concentrated hydrochloric acid to the hot filtrate, a nearly colorless precipitate of phenylanthranilic acid is thrown down. This is filtered off when cold, washed with cold water, and dried. The yield is about 13 g. The product may be further purified by recrystallization from acetic acid and alcohol.

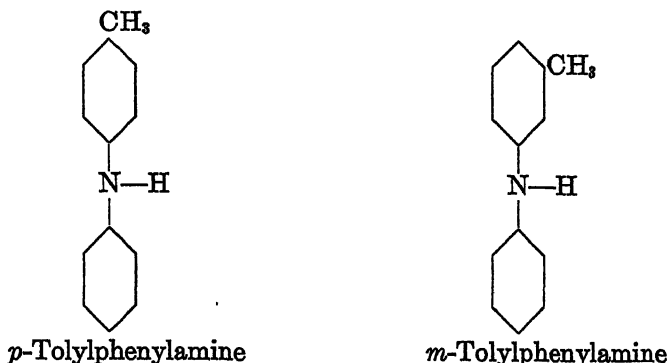
(c) *Preparation of the indicator for use:* A stock solution of the sodium salt (0.005 *M*) is prepared. The sodium salt is prepared by dissolving the acid in an equivalent quantity of sodium carbonate. Three to five drops of this solution are sufficient for most determinations.

(d) *Use of the indicator:* The color change is from colorless in the reduced form to pink in the oxidized form at an oxidation potential of 1.08 volts in sulfuric acid solutions of low acidity. Only one set of investigators has used the indicator, but they report it satisfactory for ceric sulfate and potassium permanganate titrations in sulfuric acid solutions, and fairly satisfactory for dichromate titrations. They reported using it for the determination of iron in ore, of vanadium in titanium-magnetite ore, of vanadium in pig iron and steel, and of vanadium in vanadium slags, using a modified Land and Jurz method and titrating with both ceric sulfate and potassium permanganate. The results were exceedingly accurate.

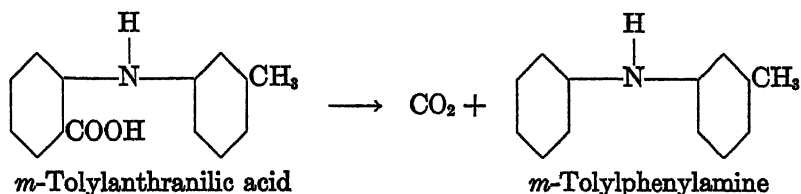
(e) *Limitations of the indicator:* The indicator has been reported completely enough to determine many of its limitations. In titration with potassium dichromate, the solutions must be at least 2 *N* in acid; also, in such titrations the color change is slow but is catalyzed by traces of ferrous iron. The color change does not seem to be any too intense, as pink is a rather pale color.

8. *p*-Tolylphenylamine and *m*-tolylphenylamine (57, 65)

(a) Structure:



(b) *Preparation of the indicator:* The basis for the preparation is the fact that, when phenylanthranilic acid is warmed to a fairly high temperature, it undergoes a decomposition in which the carboxyl group splits off carbon dioxide and the diphenylamine derivative remains. The reaction proceeds in this manner:



Specific details for preparing *m*-tolylphenylamine are as follows: A mixture of 2 g. of *o*-chlorobenzoic acid, 8 g. of *m*-toluidine, 2 g. of potassium carbonate, and about 1 mg. of precipitated copper is boiled for 2 hr. and then cooled. The brown fused mass is boiled with dilute hydrochloric acid and filtered; the filtrate is discarded. The residue is dissolved in alkali, filtered to remove traces of resin, and reprecipitated with hydrochloric acid. This material is *m*-tolylanthranilic acid and may be purified by recrystallization from benzene. The yield thus far is about 65 per cent of the theoretical. To produce the tolylphenylamine, the *m*-tolylanthranilic acid is distilled; this brings about the decomposition previously mentioned. The *m*-tolylphenylamine may be further purified by redistillation or by recrystallization from benzene.

p-Tolylphenylamine is prepared similarly, using *p*-toluidine in place of *m*-toluidine.

(c) *Preparation of the indicators for use:* The indicator solution is a 1 per cent solution in sulfuric acid. About three drops are used per determination.

(d) *Use of the indicators:* The indicators have been applied to dichromate titrations and especially to the determination of iron or chromium. The solutions should contain the usual 20 cc. of the sulfuric acid-phosphoric acid mixture, and 15 cc. of 6 *N* hydrochloric acid in a total volume of 250 cc. One drop of 0.1 *N* dichromate will produce a deep blue coloration that is easily visible and lasts for 10 to 15 min. The indicator reaction is easily reversed, and in the determination of chromium the chromic acid formed can be directly titrated with ferrous sulfate, using either indicator. The indicators may also be used in permanganate titrations.

(e) *Limitations of the indicators:* The indicators have not been reported for titrations other than ferrous ion-dichromate titrations. However, in this reaction the color development is greatly inhibited by the presence of mercury salts. The color also does not develop except in the presence of ferric ion, which leads one to suspect that the color is really due to complex formation.

9. Other indicators of the diphenylamine series (23, 27, 66)

(1) *p*-Nitrodiphenylamine: The indicator solution is a 0.01 *M* solution of the indicator in glacial acetic acid, of which two or three drops are used per determination. The indicator has been applied to ceric sulfate-ferrous sulfate titrations, but it is irreversibly oxidized by an excess of oxidant; therefore back-titrations or the reverse titrations are impossible. Dichromate-ferrous ion titrations are unsatisfactory with the indicator because of the slow development of color. The potential is high at the color change, being 1.03 volts in acid solutions.

(2) 2,4-Diaminodiphenylamine: The indicator is prepared by the reduction of the dinitro compound with tin and hydrochloric acid. The method of preparing the indicator solution has not been reported, but the indicator is probably dissolved in acetic or sulfuric acid. It has an oxidation potential at the color change of 0.70 volt in 2 *N* sulfuric acid. It has been used satisfactorily in ceric sulfate-ferrous sulfate titrations and in potassium dichromate-ferrous sulfate titrations.

The indicator has also been used satisfactorily in ceric sulfate-titanous chloride titrations, in which the reverse reaction is good. The indicator is stable toward excess oxidant and strong acids.

(3) *Two amino substitution products of diphenylamine:* 2,4-Dinitro-4'-aminodiphenylamine and 2,4-dinitro-4'-methoxydiphenylamine, when reduced with tin and hydrochloric acid, yield products having indicator properties over the same range as diphenylamine and 2,4-diaminodiphenylamine, but possessing no particular advantages over the latter.

(4) *Diphenylaminedicarboxylic acids:* 2,2', 2,3', and 2,4'-dicarboxylic acid derivatives of diphenylamine are compounds with indicator properties. The introduction of the two carboxyl groups increases the oxidation po-

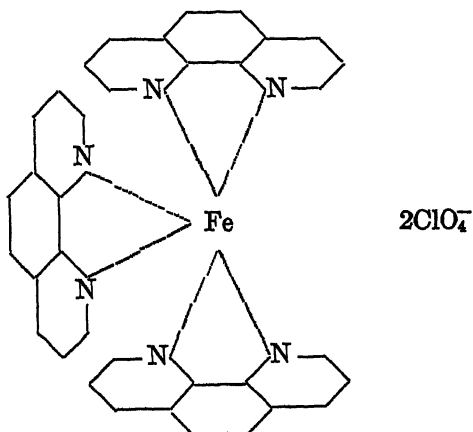
tential at the color change. The three indicators are useful in solutions that are strongly acidic and indicate the end of oxidation-reduction reactions in solutions 16 to 20 *N* in sulfuric acid.

B. COMPLEX COMPOUNDS USED AS INDICATORS

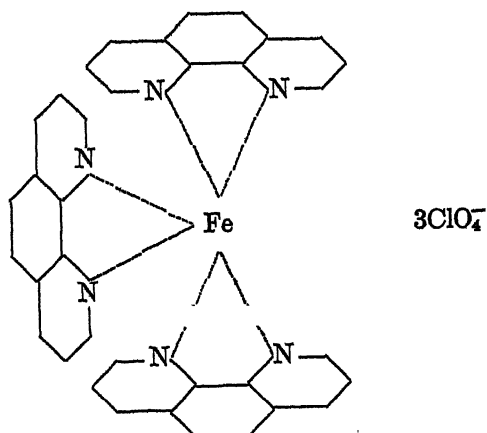
This group includes only two types of compounds,—*o*-phenanthroline ferrous ion, which is used as the sulfate or perchlorate, and nitrophenanthroline ferrous ion, which is used as the sulfate only. *o*-Phenanthroline ferrous ion is the most successful of all the oxidation-reduction indicators that have been thoroughly investigated and reported.

1. The *o*-phenanthroline ferrous complex (5, 32, 38, 53, 55, 66, 67, 69, 71, 72)

(a) Structure:



o-Phenanthroline ferrous perchlorate (red)



o-Phenanthroline ferric perchlorate (blue)

(b) *Preparation of the indicator:* To a freshly prepared mixture of 125 g. of glycerol, 125 g. of 100 per cent sulfuric acid, and 50 g. of arsenic oxide, contained in a 1-liter flask provided with a thermometer, stirrer, and reflux condenser and maintained at a temperature of 115°C., is added all at once 25 g. of *o*-phenylenediamine. The temperature rises about 10°C. The mixture is heated carefully at 140°C. for 2 hr., care being taken to avoid all higher temperatures. The material is poured into water, made strongly alkaline, and allowed to stand for at least 1 day. The tarry product is separated, washed, and dried at 110°C. until hard and brittle. The mass is pulverized and extracted with benzene in a Soxhlet until the residue no longer gives a red color with ferrous sulfate (this requires from 10 to 20 hr.). For the purification of the crude phenanthroline which crystallizes on the evaporation of the benzene, it is dissolved in dilute acid, and alkali is added until a small tarry precipitate appears; the precipitate is discarded, and the base is entirely precipitated by adding excess alkali. The solid may be recrystallized from water (with decolorizing carbon) as the monohydrate.

Smith and Getz (55) have described in detail a process for producing the indicator on a large scale, using vats holding 40 and 50 gallons of solution and employing complicated stirrers and thermostats.

(c) *Preparation of the indicator for use:* Method A (preparation of *o*-phenanthroline ferrous sulfate)—A 0.025 *M* solution of the sulfate is prepared by dissolving the stoichiometric amount of the monohydrate (14.85 g. per liter) in a 0.025 *M* ferrous sulfate solution. One drop is used in 100–150 cc. of volume for each titration.

Method B (preparation of *o*-phenanthroline ferrous perchlorate)—The 0.025 *M* solution of the sulfate is prepared as directed in method A. To a portion of this solution (100 or 1000 cc.) is added dropwise, with vigorous stirring, a dilute solution of perchloric acid until the bright red precipitate which is formed leaves, after settling, an almost colorless supernatant liquid. The colorless liquid is decanted from the precipitate as completely as possible. Centrifuging accelerates the process. The precipitate is washed four or five times with small additions of water, centrifugal separations being used to remove the excess perchloric acid. Finally the precipitate is filtered through a glass filtering crucible or funnel and dried at 80–90°C. A saturated solution of the perchlorate is prepared; it contains about 0.795 g. per liter. In each determination 1 cc. of this solution is used.

Method C (alternative method of preparing *o*-phenanthroline ferrous perchlorate)—0.94 g. of c. p. iron filings in a 300-cc. flask is dissolved by the addition of 10 cc. of 70 per cent perchloric acid that has been diluted with water to 60–75 cc. The flask is fitted with stopper and tubes so that a stream of carbon dioxide can be passed through the solution. The

liquid is heated almost to a boil, carbon dioxide being passed through the solution continuously and water being replaced as it evaporates, until the iron has completely dissolved. The solution is cooled, with the gas stream still passing through it, and is then diluted to 1000 cc. in a 1.5-liter beaker, with filtration if necessary. To 500 cc. of distilled water which has been heated to boiling is added slowly, with constant stirring, 10 g. of *o*-phenanthroline monohydrate. The ferrous perchlorate solution containing excess perchloric acid is cooled to 0°C., and the hot solution of dye base is added to it slowly with constant stirring. The mixture is allowed to cool and the solid to settle out for 30 min. The colorless supernatant liquid is poured through a 30-cc. glass filtering crucible, using reduced pressure. Finally, the red crystalline precipitate is transferred to the crucible and filtered dry. It is washed five or six times with small portions of distilled water and then dried at 80–90°C. For a stock solution, a saturated solution of the ferrous perchlorate of *o*-phenanthroline is prepared by dissolving 0.795 g. of the solid in 1 liter of water. In determinations using 0.01 *N* reagents, 1 cc. of this stock solution is employed.

(d) *Use of the indicator:* The indicator shows a color change from red in the reduced form to blue in the oxidized form at an oxidation potential of 1.20 volts in 1 *M* sulfuric acid. This corresponds to a 90 per cent oxidation of the indicator. The end point is reached with 0.01 per cent excess of oxidant. The color change is vivid and intense, as well as reversible and mobile. The indicator is non-reactive with many commonly occurring anions and cations, and is not inhibited by mercury salts. The oxidized color does not fade on standing. Most investigators report the indicator correction to be negligible, except in microanalysis.

The indicator is used in almost all ceric sulfate titrations and in many dichromate titrations. It can be used in the determination of vanadium and in the determination of vanadium in the presence of iron, chromium, or molybdenum. It is used in the titration of arsenious oxide and of potassium ferrocyanide with ceric sulfate. It may be used as the indicator in the determination of chromium and vanadium in steel by the use of potassium permanganate and perchloric acid. It can be employed when ceric sulfate is used as the titrating agent in the determination of chromium in iron ore and of iron in ferrovandium by using the Walden silver reductor or in the determination of chromium in a chromite. The indicator may be used in the standardization of ceric sulfate by means of sodium oxalate or arsenious oxide, using iodine monochloride as a catalyst. It may be used as the indicator in the ceric sulfate titration of ferrocyanide ion, thallous ion, antimonite ion, uranyl ion, hydrogen peroxide, and sodium nitrate.

In the presence of acetone and sulfuric acid, iodides may be titrated

quantitatively with ceric sulfate to a visible end point, using *o*-phenanthroline ferrous ion as the indicator. In this titration bromides interfere only slightly and chlorides less. Their effect may be almost entirely removed by diluting while keeping the acid concentration constant.

(e) *Limitations of the indicator*: The indicator is limited by its high oxidation potential to reactions producing the potential at the equivalence point. When dichromate is titrated with ferrous sulfate solution the color change requires a little time, and therefore the end point must be approached drop by drop from that direction. In the determination of iodides too little acid leads to high results and is time-consuming, while too much acid leads to low results and a fleeting end point. The indicator is not as suitable in titrations with the ceric nitrate and ceric perchlorate ions, as a momentary preferential oxidation of the indicator takes place and some time is required for the pink color to develop. Some students in the laboratories of the University of Georgia have reported that the indicator correction is not always negligible and is quite considerable in microdeterminations.

2. Nitrophenanthroline ferrous complex (23,55)

(a) *Structure*: Chemical analysis shows that the structure of this complex corresponds to the formula $C_{12}H_7N_3O_2$. The position of the nitro group has not been established.

(b) *Preparation of the indicator*: 1 g. of *o*-phenanthroline is dissolved in 10 cc. of concentrated nitric acid and the solution is added to 25 cc. of 95 per cent sulfuric acid. After 1 hr. at 115–120°C. the product is precipitated by dilution with water and neutralization with sodium hydroxide. It can probably be purified by redissolution in dilute acid, partial neutralization, filtration, and reprecipitation with alkali.

(c) *Preparation of the indicator for use*: The ferrous complex can be formed by direct reaction between the ferrous salt and the dye base. In all probability a 0.025 *M* solution is prepared by dissolving the stoichiometric proportion of the nitrophenanthroline base in 0.025 *M* ferrous sulfate, although specific quantities or concentrations are not mentioned in the reference (23), nor are the quantities to be used in each determination stated.

(d) *Use of the indicator*: There is some confusion in the literature as to just what oxidation potential is necessary to cause the color change. Hammett, Walden, and Edmonds (23) give the potential as 0.11 volt higher than the value for the phenanthroline ferrous complex under the same conditions, which would make it 1.31 volts; Smith and Getz (55) give it as 1.55 to 1.6 volts. The former say that it can be used in ceric sulfate titrations, while the latter do not agree. The color change is from red in the reduced form to greenish blue in the oxidized form, and is reversible.

According to Smith and Getz (55), the indicator is applicable to titrations using ceric perchlorate or nitrate ions, $(\text{Ce}(\text{ClO}_4)_2)^{++}$ or $(\text{Ce}(\text{NO}_3)_6)^{--}$, in the form of the free acids or of their ammonium or potassium salts; with these, arsenite and oxalate may be determined.

(e) *Limitations of the indicator:* Smith and Getz (55) state that the nitrophenanthroline ferrous complex cannot be used in sulfate titrations on account of the high oxidation potential necessary to cause the color change. The greenish blue color of the oxidized form is of low intensity. Immediate additions of ferrous sulfate to oxidized portions of the indicator regenerate the red color instantaneously, but if it is allowed to stand before the addition of the ferrous solution the color is regenerated more slowly. Apparently the ferric complex decomposes into ferric ions and nitrophenanthroline ions; the addition of ferrous ions before this reaction is complete produces the ferrous complex by reaction with the ferric complex, but later additions produce it by the action between the ferrous and the nitrophenanthroline ions.

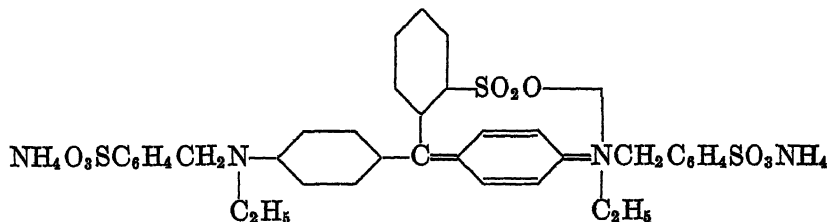
The indicator would be limited mainly by the high potential necessary to cause the color change.

C. COMMERCIAL DYES USED AS OXIDATION-REDUCTION INDICATORS

In this group an attempt has been made to include dyes that are used only under their trade names. In an article of this type it would be impossible to include all such dyes. Readers further interested in this group are referred to an article by Smith and Bliss (54) for dyes usable with bromate and to two articles by Knop (35) on commercial dyes used as indicators. The dyes from these three reports which are given in this group have been selected as the best in the opinion of the original investigator and of later writers.

1. *Erioglaucin A* (4, 19, 32, 34, 35, 36, 51)

(a) *Structure:*



Ammonium 4,4'-bis(ethyl(4-sulfobenzyl)amino)triphenylmethylsulfonate

(b) *Preparation of the indicator:* Erioglaucin A has a number of trade names, of which "Erioglaucin" is the best known. It is a patented product,

but the exact details of its manufacture are secret. However, Schultz (51) makes the following statements concerning its preparation: It is prepared by the condensation of benzaldehyde-*p*-sulfonic acid with ethylbenzylanilinesulfonic acid (perhaps with ethylbenzylaniline and additional sulfonation) to form a leucosulfonic acid, which is oxidized and then converted to the ammonium salt.

Beilstein (4) is only slightly more complete, stating that the dye is prepared by the condensation of benzaldehydesulfonic acid(2) with ethyl-(4-sulfobenzyl)aniline and oxidation of the resulting leuco compound with lead dioxide in hydrochloric acid solution.

This indicator is obtainable through the National Aniline and Chemical Company.

(c) *Preparation of the indicator for use:* A 0.1 per cent aqueous solution of the indicator is used and keeps well. From 0.5 to 1 cc. of the indicator solution is used per determination.

(d) *Use of the indicator:* The indicator has a blue color in water solution, a green color in acid solution, and at an oxidation potential of 0.71 volt is oxidized to a bluish red in acid solutions under 1.5 *N* in sulfuric acid. The indicator change is sharp, intense, and reversible.

The indicator is best applied to permanganate titrations. It is used in the titration of ferrocyanide with permanganate,—a titration having an end point which it is difficult to determine accurately without the indicator. In the standardization of permanganate against Mohr's salt the end point is much more brilliant if Erioglaucin A is used. It can be used in either sulfuric or hydrochloric acid solution. The indicator is effective in the determination of iron with permanganate, even in the presence of considerable amounts of nickel and cobalt. No correction is necessary in the iron determination. The use of phosphoric acid can be omitted, as the color change is so brilliant that it is not disturbed by the ferric ion present.

Kolthoff (28, 29) states that the indicator is not very satisfactory in ceric sulfate titrations, while Knop (34, 35) does not agree. The latter says that it is an excellent indicator for the titration of ferrous ion with ceric sulfate or *vice versa*. Furthermore, he states that the indicator works equally well in hydrochloric acid and sulfuric acid solutions, and that in the presence of calomel and of an excess of mercuric chloride the transition is very sharp. A very slight excess of ceric sulfate turns the indicator rose-red in the presence of ferric salts; in the unoxidized form it is orange or pale rose in acid solution.

(e) *Limitations of the indicator:* The indicator cannot be used with dichromate. While it can be used in hydrochloric acid solution, there are other triarylmethane dyes that perform better. The indicator in the oxidized form is rather unstable, especially in the presence of an excess

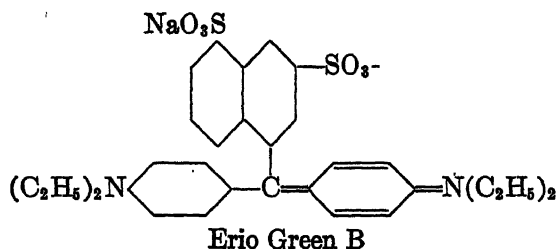
of oxidant; moreover, the color change becomes less pronounced in strongly acid media.

The titration of iron which has been reduced with zinc by means of the indicator and permanganate is not so satisfactory; the titration of iron which has been reduced with stannous chloride is better; the titration of electrolytic cadmium is best of all. The oxygen dissolved from the air interferes in microdeterminations.

In ceric titrations there is a short time interval necessary for the full color change at the end point, but the chance of overtitration is slight. In almost all titrations the end-point color fades slowly, but it lasts sufficiently long for most determinations.

2. Erio Green B (19, 32, 35, 51)

(a) Structure:



(b) *Preparation of the indicator:* This indicator is also a triarylmethane dye that is manufactured by patented processes. Schultz (51) gives three methods of preparation: (1) by the condensation of tetraalkyldiaminobenzhydroxide with naphthalene, chloronaphthalene, or their monosulfonic acid derivatives, followed by sulfonation of the resulting leuco acid and oxidation; (2) by the condensation of tetramethyldiaminobenzhydroxide with naphthalenedisulfonic acid, followed by oxidation; (3) by the condensation of *p*-alkylaminobenzaldehyde with 1 mole of alkylated xenylamine or its sulfonic acid derivative and 1 mole of an aromatic hydrocarbon or its halogen, sulfo, or halogensulfo derivatives.

(c) *Preparation of the indicator for use:* The indicator solution is a 0.1 per cent aqueous solution, of which from 0.5 to 1 cc. is used in each 150–200 cc. of titrating volume.

(d) *Use of the indicator:* The indicator is bluish green in water solution, a yellow color in acid solution, and orange colored when oxidized. The last color change takes place in acid solutions at an oxidation potential of 0.72 volt and is sharp and distinct.

The indicator may be used in the permanganate-ferrocyanide titration where the end point would not ordinarily be sharp. It can be used in the ferrous-permanganate titration to sharpen the end point; when nickel or

cobalt is present, the color change of the indicator prevails over the color of these ions. The color change is not disturbed by the ferric ions formed and the use of phosphoric acid may be omitted. In this titration the end point is determined within one drop of 0.05 *N* potassium permanganate.

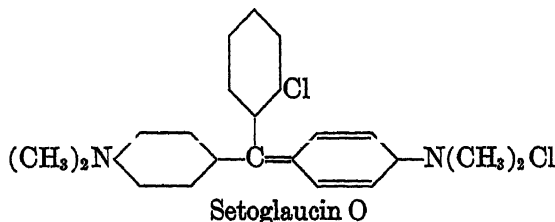
The indicator is applicable to the ceric-ferrous titration or *vice versa*. No blank correction is necessary. A very slight excess of ceric sulfate turns the indicator a rose color, which in the presence of ferric salt becomes orange. The indicator is equally satisfactory in hydrochloric and sulfuric acid solutions, and in the presence of mercury salts.

(e) *Limitations of the indicator*: The indicator cannot be used in dichromate titrations. It is less sensitive in the permanganate-ferrous titration than is Erioglaucin A. As with Erioglaucin A, Erio Green B is rather unstable in the oxidized form, especially in the presence of excess of oxidant; also, the color change becomes less pronounced as the acid concentration increases. *

In the ceric titrations the same difficulties are encountered with Erio Green and Erioglaucin.

3. Setoglaucin O (35, 51)

(a) *Structure*:



(b) *Preparation of the indicator*: Setoglaucin O is also a patented dye-stuff. Schultz gives its preparation as follows: The dye is prepared by the condensation of *o*-chlorobenzaldehyde with dimethylaniline and oxidation of the resulting tetramethyldiamino-*o*-chlorotriphenylmethane.

The indicator is sold by the National Aniline and Chemical Company.

(c) *Preparation of the indicator for use*: The stock solution is a 0.1 per cent aqueous solution and 0.5–1 cc. is used per determination in each 150–200 cc. of titrating volume.

(d) *Use of the indicator*: In neutral solutions the dye has a blue color, which becomes yellow in acid solutions. The dye is oxidized to a yellow red in acid solutions at a potential of 0.72 volt. The indicator works in either hydrochloric or sulfuric acid solution; the solution should be about 2 *N* with respect to acid.

Knop (35) reports, "the sensitiveness of the indicator, the convenience

of the color change potential for the iron titration, as well as its resistance to the oxidative action of permanganate, make it ideal for the micropermanganate titration of iron. The error in determining 1 mg. of iron is less than 0.1 per cent (of the result)."

Knop further reports that the indicator is unaffected by the presence of tin and mercury salts, and that it is especially useful in determining iron in the presence of nickel or cobalt, although it produces a color change not greatly different from that of the solution, which is sharp and distinct. The indicator is reported as being used in the standardization of permanganate against oxalic acid or sodium oxalate, without any correction, after the usual preliminary titration.

Knop (35) reports that the indicator is the best of the triarylmethane dye indicators in hydrochloric acid solution.

(e) *Limitations of the indicator:* While accurate results can be obtained with the indicator over wide ranges of hydrochloric acid concentration, best results are obtained when this concentration is low. Unfortunately, very little has been accomplished toward applying the indicator to determinations other than permanganate determinations of iron, but it is possible that it has the same weakness as the other triphenylmethane dyes,—namely, lack of resistance to excess oxidant, inapplicability to dichromate titrations, and poor color change in strongly acid solutions

4. Cyanine B (35, 36, 51)

(a) *Structure:* The structure of the dye has not been established.

(b) *Preparation of the indicator:* Cyanine B is prepared from Patent Blue V.

Schultz (51) gives for the preparation of Patent Blue V the following methods: (1) Reduction of the *m*-nitrotetraethyldiaminotriphenylmethane resulting from the condensation of *m*-nitrobenzaldehyde with 2 moles of diethylaniline, and the conversion of the amino compound thus formed to the hydroxy compound by diazotization. The last-named product is sulfonated, converted to its calcium, magnesium, or sodium salt, and oxidized.

(2) Condensation of *m*-hydroxybenzaldehyde with diethylaniline and treatment of the resulting hydroxy compound, which is identical with that obtained in method 1, as described above.

Schultz gives the following preparation of Cyanine B from Patent Blue V: "Oxidation of Patent Blue V with chromic acid et cetera, whereby a C_2H_5 group is probably joined on."

(c) *Preparation of the indicator for use:* A 0.1 per cent aqueous solution is prepared, and from 0.5 to 1.0 cc. is used per 150–200 cc. of final titrating volume.

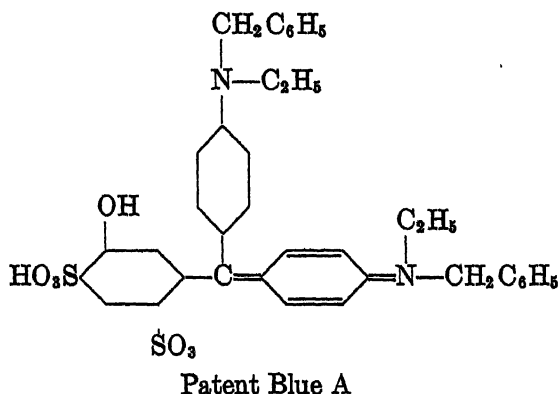
(d) *Use of the indicator:* At an oxidation potential of 0.73 volt in a solution which is 1 *N* in either hydrochloric or sulfuric acid the indicator shows a color change. In sulfuric acid the color change is from yellow in the reduced form to orange in the oxidized form; in hydrochloric acid solution the color change is from yellow to brownish orange. The color change is reversible in both cases to a greenish yellow. The color change is sharp and the end-point color is long lasting.

The indicator has been applied only to sharpening the end point in the permanganate-ferrous titration. It is very satisfactory for this, producing accurate results in either hydrochloric acid or sulfuric acid solution, even in the presence of considerable nickel or cobalt. It is especially good in microdeterminations.

(e) *Limitations of the indicator:* The indicator cannot be applied to dichromate titrations, and is probably not resistant to excess oxidant. It is not satisfactory for use in the presence of zinc. However, tin and mercury salts do not affect the end point or the color development. Care must be taken either to remove dissolved oxygen or to carry out the titration in an atmosphere of carbon dioxide.

5. Patent Blue A (35, 51)

(a) *Structure:*



(b) *Preparation of the indicator:* Schultz gives the following information concerning the manufacture of the dye: (1) The *m*-nitro compound resulting from the condensation of *m*-nitrobenzaldehyde with 2 moles of ethylbenzylaniline is reduced, and the amino compound thus formed is converted to the hydroxy compound by diazotization. The product is sulfonated, converted to the calcium compound, and oxidized.

(2) *m*-Hydroxybenzaldehyde is condensed with ethylbenzylaniline and

the resulting hydroxy compound (which is identical with that described in method 1) is worked up by the procedure described in method 1.

This dye is available through the National Aniline and Chemical Company.

(c) *Preparation of the indicator for use:* A 0.1 per cent aqueous solution is prepared, and from 0.5 to 1.0 cc. is used per determination in 150–200 cc. of final volume.

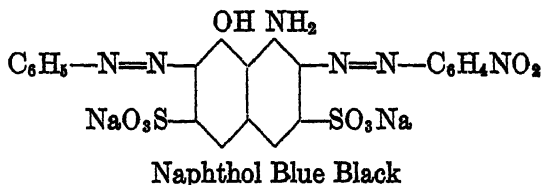
(d) *Use of the indicator:* At an oxidation potential of 0.71 volt in a solution which is 1 *N* in either hydrochloric or sulfuric acid the indicator shows a change from a greenish yellow color in the reduced form to a rose color in the oxidized form. The color change is very sharp and easily reversed.

The indicator has been applied only to sharpening the end point in titrations of iron with permanganate. It is very satisfactory for this, producing accurate results in both hydrochloric acid and sulfuric acid solutions.

(e) *Limitations of the indicator:* The indicator cannot be applied to dichromate titrations; hence one may conclude that it is not resistant to an excess of oxidant. In fact, Knop (35) states that the end color fades rapidly, so that the first alteration of color indicates the equivalence point.

6. Naphthol Blue Black (18, 51, 54)

(a) *Structure:*



(b) *Preparation of the indicator:* Schultz (51) gives the following information regarding its preparation: 13.8 kg. of *p*-nitroaniline is diazotized and the product is combined with 32 kg. of H acid (1-amino-8-naphthol-3,6-disulfonic acid) in acid solution. The solution is made faintly alkaline with soda and into it is led the solution resulting from the reaction between 9.3 kg. of aniline and diazobenzyl chloride. The precipitated dye is filtered off and dried.

(c) *Preparation of the indicator for use:* 0.2 cc. of a 0.2 per cent water solution is used to each 100 cc. of titrating solution.

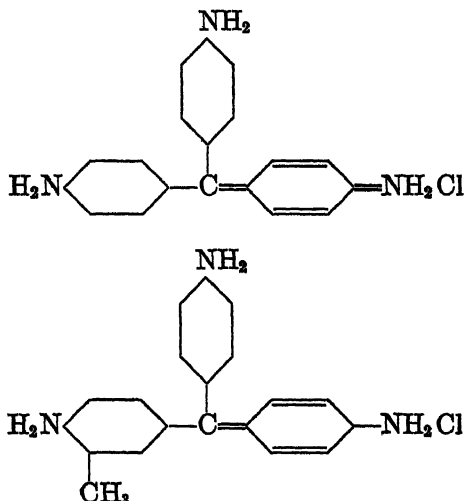
(d) *Use of the indicator:* Smith and Bliss (54) report the dye as being quite satisfactory in bromate determinations under ordinary conditions. This must mean that fairly weak acidities and room temperature are necessary. The color change is from bright blue to colorless and is very

sharp. As the indicator can be used with bromate, it is applicable to the determination of many metals by means of 8-hydroxyquinoline.

(e) *Limitations of the indicator:* The indicator is irreversibly oxidized by an excess of oxidant. It is not satisfactory in strongly acidic media.

7. Fuchsin (7, 51, 54)

(a) *Structure:* The commercial dye is a mixture of the two chemical compounds below.

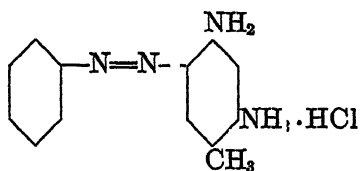


(b) *Preparation of the indicator:* Schultz (51) gives the following methods of preparing the dye: (1) A mixture of aniline, *o*- and *p*-toluidines, and their hydrochloride salts is heated with nitrobenzene or nitrobenzene and nitrotoluene in the presence of iron and zinc chloride. (2) A mixture of aniline, *o*-toluidine, and *p*-toluidine is heated with arsenic acid.

(c) *Preparation of the indicator for use:* A stock solution is prepared by dissolving 2 g. in 1 liter of water, and 0.2 cc. of this solution is used for each 100 cc. of final volume.

(d) *Use of the indicator:* The indicator is recommended for the determinations using bromate as the titrating agent at low temperature. The color change is from a reddish yellow to a lavender. Using the indicator, the investigators were able to determine 100 mg. of arsenic or antimony with an error of only 0.2 per cent; mercuric salts were used to guide the reaction.

(e) *Limitations of the indicator:* The indicator is oxidized from the lavender color to colorless by an excess of reagent and the reaction is irreversible. It is not applicable to high temperatures or to titrations in strongly acid solutions.

8. *Chrysoidine R* (13, 32, 51, 54)(a) *Structure:*

Chrysoidine R

(b) *Preparation of the indicator:* 20 g. of aniline is dissolved in a solution of 63 g. of hydrochloric acid in 1.5 liters of water. This is cooled with 500 g. of ice and diazotized with a solution of 14.8 g. of sodium nitrite (100 per cent) in 1 liter of water. To this solution is added slowly a solution of 26 g. of 4-*m*-tolylenediamine in 250 cc. of 10 per cent hydrochloric acid. The liquor turns faintly reddish orange but scarcely any dye separates. After stirring for 15 min., a dilute solution of sodium acetate (about 1 *N*) is run in slowly; this causes a much richer color to develop. Not too much acetate solution should be allowed to run in, only enough to bind the excess acid; addition of the acetate should be stopped while the solution is still weakly acidic with mineral acid. The reaction mixture is stirred for at least 1 to 2 hr. and as much longer as it takes for the diazo compound to disappear. The liquid is heated to boiling and filtered; 250 g. of solid common salt is added and the liquid is again heated to boiling until the jelly-like precipitate first formed changes to a crystalline one. The solid dye is filtered off by suction and dried.

(c) *Preparation of the indicator for use:* A 0.2 per cent aqueous solution is prepared. Two or three drops of this are sufficient in a volume of 100 cc.

(d) *Use of the indicator:* The indicator is useful in bromate titrations where a color change from reddish yellow to colorless is desired. Kolthoff and Sandell (32) give it as a preferred indicator in bromate titrations. Smith and Bliss (54) report that the color is bleached by reducing agent, is restored at the end point, and is then destroyed by an excess of oxidant.

(e) *Limitations of the indicator:* The indicator is destroyed by excess oxidant and has not been applied to titrations other than the bromate titration.

9. *Brilliant Ponceau 5R and Bordeaux* (13, 32, 54)

(b) *Preparation of the indicators:* The preparation of the indicators is not given by Schultz, but can probably be found in reference 13. Both dyes can be obtained from most dye manufacturers.

(c) *Preparation of the indicators for use:* A 0.2 per cent aqueous solution is used as the indicator solution.

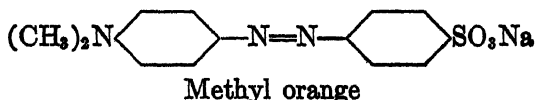
(d) *Use of the indicators:* Both are applicable to bromate titrations. Brilliant Ponceau 5R is recommended by Smith and Bliss (54) as being very advantageous because it resists fading and reacts moderately rapidly at room temperature and low acidities, and the color change is accelerated by increased temperatures and acidities. The best conditions are a normality of about 1.0 with respect to hydrogen ion and a temperature of 50–60°C. With Ponceau the color change is from scarlet red to colorless.

Kolthoff and Sandell (34) prefer Bordeaux in bromate titrations. Smith and Bliss discuss the dye, saying that the color change is from red to colorless and that the dye is suitable at moderate pH and low temperatures.

(e) *Limitations of the indicators:* Both indicators are irreversibly oxidized by an excess of oxidant.

10. Methyl orange (10, 32, 44, 63, 73)

(a) *Structure:*



(b) *Preparation of the indicator:* 10 g. of sulfanilic acid is dissolved in a solution of 2.5 g. of anhydrous sodium carbonate in 100 cc. of water. A solution of 3.5 g. of sodium nitrite in 20 cc. of water is then slowly added. The mixture is cooled in ice, a solution of 6 cc. of concentrated hydrochloric acid in 20 cc. of water is poured into it, and the liquid is made alkaline with caustic soda. The separation of methyl orange begins at once and is assisted by the addition of about 20 g. of common salt. The precipitate is filtered by suction and recrystallized from hot water. The yield is almost theoretical.

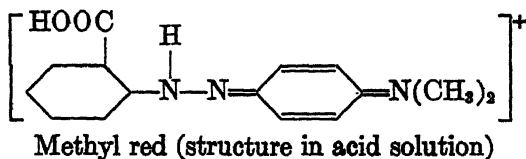
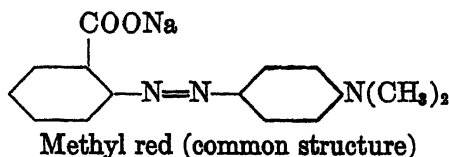
(c) *Preparation of the indicator for use:* A 1 per cent aqueous solution is prepared, and one or two drops of this are used per determination.

(d) *Use of the indicator:* The indicator is best applied to bromate titrations. It is irreversibly oxidized in such cases, however. It is the indicator commonly used in the determination of arsenite and antimonite by means of bromate. Methyl orange may be satisfactorily used as the indicator in the determination of trivalent antimony with ceric sulfate, whereby it is irreversibly oxidized.

(e) *Limitations of the indicator:* The main fault of the indicator is that it is not sufficiently stable toward an excess of oxidant to be used with the stronger oxidants. Even with bromate it is irreversibly oxidized. The color change with methyl orange is not good and is hindered by high concentrations of acids.

11. Methyl red (14, 19, 20, 31, 32, 44, 45, 70, 76)

(a) Structure:



The structure of the violet-colored compound has not been determined.

(b) *Preparation of the indicator:* Method A—20 g. of anthranilic acid is dissolved in 50 cc. of 2 *N* sodium hydroxide and a solution of 8 g. of sodium nitrite in 100 cc. of water is added. The mixture is cooled in ice and poured into 50 cc. of 2 *N* hydrochloric acid. The solution so obtained is mixed with a prepared solution of 12 g. of dimethylaniline in 100 cc. of 1 *N* hydrochloric acid. The mixture is made distinctly alkaline with sodium hydroxide. The sodium salt separates shortly and is filtered by suction. If desired, it can be recrystallized from water.

Method B—12 g. of dimethylaniline is dissolved in a suspension of 20 g. of anthranilic acid in 100 cc. of water, the mixture is cooled in ice, the sodium nitrite mixture described in method A is added, and the dye is precipitated.

Conant (14) describes in detail a method of making the dye base in large quantities.

(c) *Preparation of the indicator for use:* 0.2 g. of methyl red is dissolved in 100 cc. of 1.2 *N* sulfuric acid. Two drops of this solution are used in most titrations. In bromate titrations a 1 per cent aqueous solution is used.

(d) *Use of the indicator:* The indicator may be used in certain ceric sulfate titrations. In the titration of ceric sulfate with ferrous sulfate, the color change is from yellowish to violet. The indicator may be used in the titration of a fluoride ion solution with thallium salts, using a modified Willard and Winter (70) method. Methyl red can be used as the indicator in the titration of trivalent antimony with ceric sulfate, and in determining hydroquinone by using ceric sulfate as the titrating agent. In the ferrous sulfate-ceric sulfate titration the indicator is satisfactory in both sulfuric and hydrochloric acid solutions and the color change is more rapid than

that of diphenylamine. It is not affected by the presence of mercury or tin salts. The color change is reversible.

The indicator may be used in bromate titrations, but it is irreversibly oxidized by the reagent. However, in spite of this it is quite satisfactory if the temperature is kept at about 40–50°C. and the acid concentration between 0.7 and 1.0 *N* with hydrochloric acid. It is sensitive to 2 or 3×10^{-5} *N* bromate.

(e) *Limitations of the indicator:* The indicator is not stable to excess oxidant, as bromate, permanganate, and chlorine all oxidize it beyond the reversing point. It is not as satisfactory in the ceric sulfate–hydroquinone titration as is diphenylamine. There is a slight indicator correction in its use. Apparently there are two separate color changes, one from deep orange to violet in ferrous–ceric titrations and the other from orange to yellow in the ceric–hydroquinone titration.

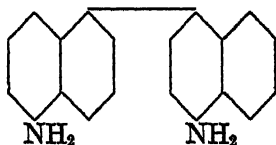
D. MISCELLANEOUS COMPOUNDS USED AS OXIDATION–REDUCTION INDICATORS

In this group are included all reported indicators that did not fall in either of the other three groups. It does not include any very widely used indicators but does include some that show promise.

The indigophenols and their substitution products are not given, since their oxidation potentials at the color change are too low to be of value at present in analytical work. Readers who are investigating indicators that change color at low potentials are referred to the work of Clark and his coworkers (8, 9) and the work of L. Michaelis (40).

1. Naphthidine (12, 16, 57)

(a) *Structure:*



Naphthidine

(b) *Preparation of the indicator:* The first workers with the compound used the method of Cumming and Steel (16), which is method 1 below, but later workers prepared it by method 2, which is the preferred method.

Method 1—20 g. of α -nitronaphthalene, 175 cc. of 90 per cent alcohol, 50 cc. of water, and 40 g. of ammonium chloride are heated together at 70°C. on the water bath; the nitronaphthalene goes into solution, but some of the ammonium chloride remains undissolved. To this is added

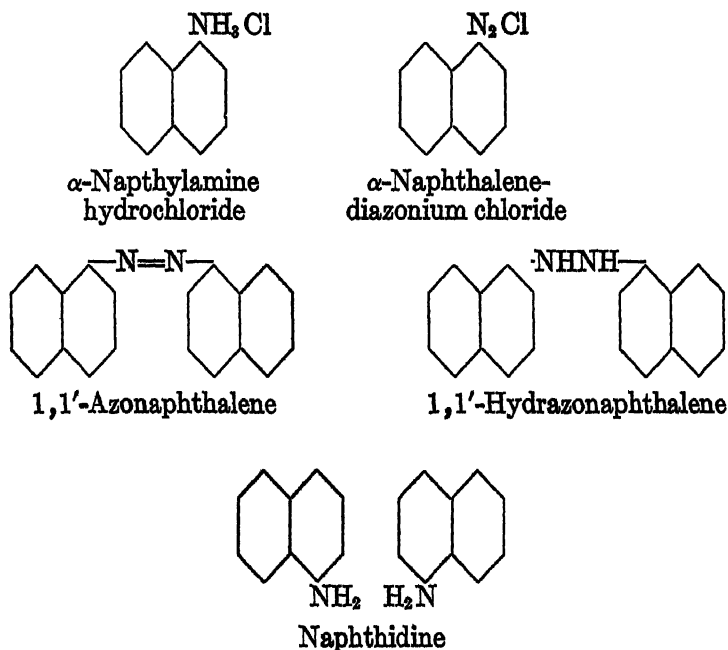
28 g. of zinc dust at such a rate that the temperature remains at 70–75°C. The hot solution, which gradually changes from red to yellow, is filtered, the residue is extracted with 90 per cent alcohol, and the combined filtrate and extract are cooled. The crystalline precipitate is removed, and a further deposit is obtained by concentrating the filtrate under reduced pressure. The α -azoxynaphthalene is purified by washing with warm water and 90 per cent alcohol. It is then dissolved in glacial acetic acid and reduced with stannous chloride in hydrochloric acid. Reduction takes place easily. Concentrated hydrochloric acid is added and the product is dissolved in water. Treatment with sodium hydroxide causes the dye to crystallize. It may be recrystallized from alcohol in white silvery plates.

Method 2—35 g. of α -naphthylamine hydrochloride is stirred into 500 cc. of water, 17.5 cc. of concentrated hydrochloric acid is added, the mechanical stirrer is started, and the solution is cooled on the ice bath to 0°C. Cold dilute sulfuric acid (21 cc. of acid of sp. gr. 1.84 plus 200 cc. of water) is then stirred in. The suspended amine salt is diazotized by slowly adding a cold solution of 14 g. of sodium nitrite in 80–100 cc. of water. The reddish brown mixture is allowed to stand for 5 min. in an ice bath, and filtered by suction, the filtrate being received in a precooled flask surrounded by an ice bath. The cold filtrate is transferred to a 2-liter beaker (ice bath), the stirrer is started, and a cold solution of 66 g. of sodium acetate (anhydrous) in 300 cc. of water is slowly added, the temperature being kept between 0° and 5°C. A cooled solution of 31 g. of sodium sulfite in 200 cc. of water is then run in slowly; a vigorous evolution of nitrogen ensues, and 1,1'-azonaphthalene begins to separate. After the addition of sodium sulfite, the stirring is continued for 5 min. The suspension is then taken out of the ice bath and warmed on the water bath. The tan or orange precipitate is filtered off and dried on a porous plate.

Twenty grams of the crude moist filter cake of 1,1'-azonaphthalene is suspended in 200 cc. of ethanol, and the mixture is brought to a weak boil. A solution of 40 g. of stannous chloride in 100 cc. of concentrated hydrochloric acid is slowly run in, with occasional stirring, until the suspended solid has turned light tan. The heating is immediately discontinued, the suspension is cooled to room temperature, and 100 cc. of concentrated hydrochloric acid is added to precipitate the rest of the naphthidine hydrochloride. Under no conditions must the solution be heated with the acid, since the dinaphthylene (or 1,1'-diamino-2,2'-dinaphthyl) present in the solution, when heated with hydrochloric acid, loses ammonia, forming dinaphthocarbazole, which precipitates with the naphthidine hydrochloride and greatly impedes its purification.

The naphthidine hydrochloride is removed from the suspension, sucked as dry as possible, and suspended in 200 cc. of water; 20 per cent sodium hydroxide solution is then added in slight excess. The mixture is kept warm for 10 min. at 40°C. with frequent stirring. The crude naphthidine is filtered, washed with water until free of alkali, and sucked as dry as possible. The crude base is boiled with 120 cc. of ethanol, and pyridine (40-45 cc.) is slowly run into the boiling suspension until the solid has dissolved. The solution is filtered hot. The filtrate is allowed to cool slowly; the naphthidine separates in well-formed crystals.

The equations for the synthesis given under method 2 are as follows:



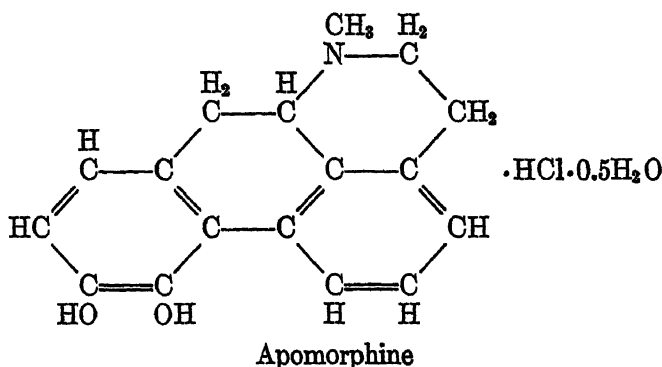
(c) *Preparation of the indicator:* The stock solution is a 1 per cent solution of the indicator in sulfuric acid. Three drops of this solution per 250 cc. give the color change.

(d) *Use of the indicator:* The indicator has been applied to the ferrous-dichromate titration only and works excellently for either direction. It gives a deep red with a slight excess of dichromate, and the color is sharply discharged by ferrous ion. Mercury salts do not interfere in the least. The deep red color is in sharp contrast to the green color of chromium salts, much more so than the blue color of most of the diphenylamine derivatives.

(e) *Limitations of the indicator:* The indicator has not been applied to titrations other than that of ferrous-dichromate. The color at the end point lasts only for 1.5 to 2 min. and then fades; this would make it of little value in permanganate titrations, as permanganate alone does this.

2. Apomorphine (39, 42, 62)

(a) *Structure:*

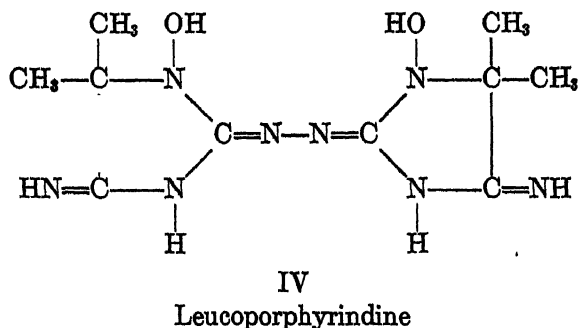
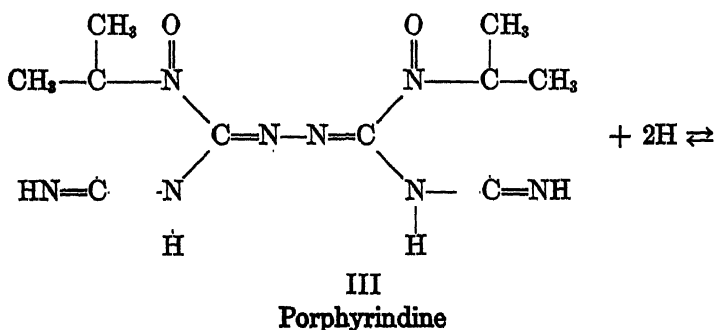
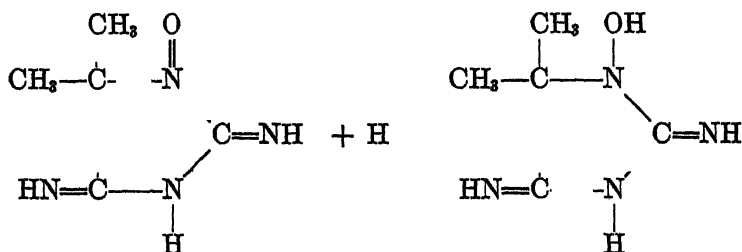


(b) *Preparation of apomorphine:* Apomorphine hydrochloride is obtained from morphine by heating it with hydrochloric acid in sealed tubes.

(c) *Preparation of the indicator for use:* A 0.1 per cent aqueous solution of apomorphine hydrochloride is used for the indicator solution; 0.3 cc. of this is used per determination.

(d) *Use of the indicator:* The indicator is used for the determination of antimony with potassium bromate. Small quantities of the indicator in hydrochloric acid solution are colored rose by free bromine, and the solution is decolorized when potassium antimonyl tartrate is added. The reaction is reversible. The same color change is produced by an antimonite with the indicator and potassium bromate. The results agree with potentiometric titrations.

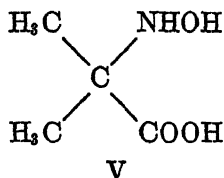
(e) *Limitations of the indicator:* The indicator can be used only with potassium bromate. *Merck's Index* (39) reports the compound as being very unstable to even moderate oxidation. It cannot be used in strong acid solutions, and the temperature must be kept between 45° and 50°C. for accurate results. Near the end point of the titration 10–20 sec. must elapse between the addition of each drop of standard solution to see if the color develops. Too much apomorphine disturbs the sharpness of the end point.

3. *Porphyrezide-porphyrizine and porphyrindine-leucoporphyrindine*(a) *Structures of the two indicators and their indicator reactions:*

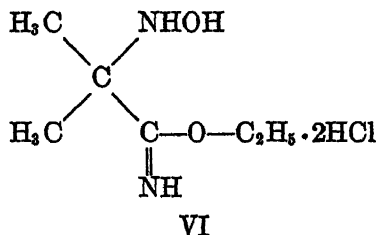
Compounds I and III are very unusual in that they have a nitrogen atom with four valence bonds in an organic compound. These equations are given by both sets of investigators. Undoubtedly I and III are theoretical compounds that cannot be isolated, but are the most likely modifications of compounds II and IV that differ from the latter only by the hydrogen ions that are present in the solutions.

(b) *Preparation of porphyrezide and of porphyrindine:* A mixture of 180 g.

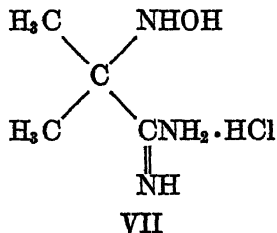
of acetoxime, 150 g. of 70–80 per cent hydrocyanic acid, and 30 g. of water is stirred for 2 days at 4°C. The temperature is raised to 15°C. and the stirring is continued for 2 more days. The product formed is α -hydroxyl-aminoisobutyric acid and, according to Kuhn and Franke (37), has the following structural formula:



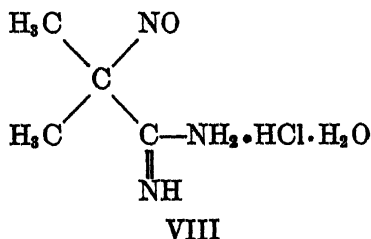
The next product in the synthesis is given by Kuhn and Franke as prepared by the method of Piloty (one of these references was not available). Piloty's directions for the preparation of the hydrochloride (VI) are as follows: The α -hydroxylaminoisobutyric acid is crystallized from ether. About 5 g. of it is suspended in 50 g. of absolute alcohol, and, while stirring and keeping the temperature at 0°C., well-dried hydrogen chloride is led in until the solution is saturated. The hydrochloride (VI) is formed on standing, the yield being almost theoretical. Evaporation is carried out *in vacuo*.



Ten grams of the above salt is dissolved in 50 cc. of absolute alcohol and 28.5 cc. of 4 *N* alcoholic ammonia (2.5 moles) is added. This requires about 4 hr. with constant shaking at 15–20°C. The ammonium chloride formed is filtered off by suction and the alcoholic filtrate is evaporated *in vacuo*. The product so formed, according to Kuhn and Franke, has the following structure:



Seven grams of the hydroxylamino amide is dissolved in 40 cc. of water containing 1 cc. of concentrated hydrochloric acid. Chlorine gas is led in slowly (about two bubbles per second) until the white nitroso material deposited on the bottom fails to increase; on the top of the supernatant chlorine solution a violet colored oil is noticed. The product formed is α -nitrosoisobutyric-amido hydrochloride and has the following structural formula:



The condensation of compound VIII with potassium cyanide takes place between very narrow temperature limits. Over a period of 2 min. 2.0 g. of α -nitrosoisobutyric-amido hydrochloride is added in small portions to a solution of 1.00 g. of potassium cyanide in 3.5 cc. of water, at a temperature of 55–60°C. The mixture is kept at this temperature for 1 more min., with constant stirring, and then cooled immediately with ice water. The crystals can be separated from the mother liquor on a porous plate or the whole can be brought to dryness over phosphorus pentoxide in a vacuum. In the latter case the mass is extracted with alcohol, thus leaving the potassium chloride on the filter. On evaporation of the alcohol, the porphyraxide separates as fine white needles.

The leucoporphyrindine can be prepared from the porphyraxide by treatment with hydrazine, using 0.5 mole of hydrazine for each mole of porphyraxide (for details see Piloty and Vogel (43)).

(c) *Preparation of the indicators for use:* The method of preparation has not been determined. The indicators are soluble in alcohol and probably in mineral acids.

(d) *Use of the indicators:* The porphyraxide–porphyraxine system shows a color shift from the red in the reduced form to colorless in the oxidized form at a potential of 0.97 volt at pH 7 and 1.34 volts at pH 0.

The porphyrindine–leucoporphyrindine system shows a color shift from blue in the reduced form to colorless in the oxidized form at an oxidation potential of 0.81 volt at pH 7 and 1.2 volts at pH 0.

The indicators have not as yet been applied to titrations.

(e) *Limitations of the indicators:* The limitations are unknown, but is is probable that the indicators are unstable toward an excess of oxidant.

4. Some inorganic indicators (59, 60, 61, 63, 64)

(1) *Sodium bromoaurate and sodium bromoaurite.* Sodium bromoaurite (NaAuBr_2) gives a colorless aqueous solution, but sodium bromoaurate (NaAuBr_4) dissolves in water with a deep yellow color. Either compound is suitable as an indicator in the determination of arsenites with ceric sulfate; they give sharp end points and can successfully replace iodine monochloride.

The yellow compound is easily reduced to the colorless one and free bromine oxidizes the colorless one to the colored. Either can be used as the indicator in the determination of antimony with bromate. The results are good.

(2) *Manganese salts.* Manganese salts may be used as catalyzers and indicators in the oxidation of oxalates with ceric sulfate.

(3) *Phosphomolybdic acid.* Hydrazine sulfate solutions react with phosphomolybdic acid to give a deep blue reduction product of molybdic acid. The addition of oxidizing agent causes the disappearance of the blue color as a result of the oxidation of the molybdenum to the hexavalent state; this reaction is reversible. The compound may therefore be used as an indicator in the titration of hydrazine salts with potassium bromate. The solution to be titrated may contain the equivalent of 10–30 cc. of 0.1 *N* hydrazine salt. It is diluted with water to about 40 cc.; 0.3 g. of powdered sodium molybdate and 10 cc. of 25 per cent phosphoric acid are then added. The solution is heated to 60–80°C. and titrated with standard potassium bromate solution until the solution becomes colorless within 30–40 sec. after the addition of the last drop. Potentiometric titrations which have been performed show the results to be reliable.

(4) *Osmic acid.* A solution of arsenite in dilute sulfuric acid gives a deep blue-black color when treated with a little osmic acid. The color is believed to be caused by colloidal osmium. The solution becomes colorless on the addition of potassium bromate. The procedure is to make the solution slightly acid with sulfuric acid, to add 0.25 g. of potassium bromide and 2 cc. of 0.25 per cent osmic acid, and to titrate with standard bromate. In the above determination the total volume should be increased if the amount of arsenic is large.

(5) *Selenium.* Potassium bromate titrations of arsenious arsenic require no indicator in bright sunlight. Methyl orange is ordinarily used. However, selenious acid may be used as follows: 5 g. of sodium bromide is added to the arsenite solution, the solution is diluted to 35 cc., 25 cc. of concentrated hydrochloric acid is added, the solution is heated to 55–60°C., 1 cc. of 1 *M* selenious acid is added, and the titration is performed. When the selenious acid is added, dark red colloidal selenium is formed

TABLE 1

Compounds reported as unsatisfactory for use as oxidation-reduction indicators

COMPOUND

1a. Substitution products of diphenylamine

1	<i>p</i> -Acetylaminodiphenylamine	Is not stable enough against excess oxidant to warrant its use
2	<i>p</i> -Aminodiphenylamine	Is easily destroyed by excess oxidant
3	2,4,6-Triaminodiphenylamine	Has no indicator properties
4	2,4,6,3'-Tetraaminodiphenylamine	Has no indicator properties
5	2,4,2',4'-Tetrabromodiphenylamine	Has no colored oxidation products
6	2,4-Dichlorodiphenylamine	Has no colored oxidation products
7	2,4,2',4'-Tetrachlorodiphenylamine	Has no colored oxidation products
8	2,4,6,2',4',6'-Hexachlorodiphenylamine	Has no colored oxidation products
9	<i>m</i> -Nitrodiphenylamine	Has no colored oxidation products
10	2,4-Dinitrodiphenylamine	Requires too high an oxidation potential for ceric ion, permanganate ion, or sodium bismuthate to cause a visible color change
11	2,4-Dinitro-4'-aminodiphenylamine	Requires too high an oxidation potential for ceric ion, permanganate ion, or sodium bismuthate to cause a visible color change
12	2,4-Dinitro-4'-methoxydiphenylamine	Requires too high an oxidation potential for ceric ion, permanganate ion, or sodium bismuthate to cause a visible color change
13	2,4,6-Trinitrodiphenylamine	Requires too high an oxidation potential for ceric ion, permanganate ion, or sodium bismuthate to cause a visible color change
14	2,4,6,3'-Tetranitrodiphenylamine	Requires too high an oxidation potential for ceric ion, permanganate ion, or sodium bismuthate to cause a visible color change
15	Sodium methyldiphenylaminesulfonate	Produces a long range of intermediate colors preceding the development of the full red, and the establishment of an end point is obscured
16	Sodium ethyldiphenylaminesulfonate	Produces a long range of intermediate colors preceding the development of the full red, and the establishment of an exact end point is thereby obscured

1b. Substitution products of diphenylbenzidine

17	2,4-Diaminodiphenylbenzidine	Is too easily destroyed by an excess of oxidant
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TABLE 1—*Continued*

NO.	COMPOUND	COMMENT
1b. Substitution products of diphenylbenzidine—<i>Concluded</i>		
18	<i>N,N</i> -Dimethyl- <i>N,N'</i> -diphenylbenzidine	Although it gives a colored oxidation product, is of too little merit to warrant its use
19	2,4-Dinitrodiphenylbenzidine	Requires too high an oxidation potential for ceric ion, permanganate ion, or sodium bismuthate to cause a visible color change
2. Complex compounds		
20	Cadmium complex of phenanthroline	Has no color in either the oxidized or the reduced state
21	Chromium complex of phenanthroline	Has a low color intensity and the color contrast is not great at the end point
22	Cobalt complex of phenanthroline	Has no color in either the oxidized or the reduced state
23	Copper complex of phenanthroline	Has no color in either the oxidized or the reduced state
24	Ferrous dipyridyl complex	Reacts too rapidly with acids, and its potential is not different from that of the ferrous phenanthroline complex
25	Nickel complex of phenanthroline	Has no color in the oxidized or the reduced state
26	Zinc complex of phenanthroline	Has no color in the oxidized or the reduced state
3. Commercial dyes		
27	Acid Green GV	Is strongly oxidized by an oxidant and the end-point color fades rapidly
28	Acid Violet 5B	Has an irreversible indicator reaction and is unstable toward an oxidant
29	Acid Violet 6 BN	Is rapidly oxidized by an oxidant and the indicator reaction is not reversible
30	Acid Violet 7B	Is rapidly attacked by an oxidant and the indicator reaction is only weakly reversible
31	Fast Acid Blue B	Is strongly attacked by an oxidant and gives a color change too soon
32	Fast Green Extra	Is partially oxidized as the oxidant is added, and the end color fades rapidly

TABLE 1—*Concluded*3. Commercial dyes—*Concluded*

33	Methyl Violet B	Is strongly attacked as an oxidant is added; the color vanishes quickly and is not reversible
34	Turkish Blue BB	Is not very resistant to an oxidant and the end color fades rapidly
35	Victoria Blue B	Produces a fleeting end-point color and is strongly attacked as an oxidant is added
36	Victoria Blue 4R	Is very strongly attacked as an oxidant is added
37	Wool Green S	Produces an end color that fades too rapidly

4. Miscellaneous compounds

38	Benzidine	Gives too many colors on oxidation to establish an end point
39	<i>N,N'</i> -Dimethylbenzidine.	Produces color changes that are not striking enough to warrant its use
40	Benzidinemonosulfonic acid	Produces color changes that are not striking enough to warrant its use
41	Dinaphthocarbazole	Produces color changes that are not striking enough to warrant its use
42	Chromotropic acid	Has no colored oxidation products
43	1-Naphthylamine	Has no colored oxidation products
44	β,β -Dinaphthylamine	Has no colored oxidation products
45	<i>m</i> -Tolyl-1-naphthylamine	Has no colored oxidation products
46	<i>o</i> -, <i>m</i> -, and <i>p</i> -Tolyl-2-naphthylamines	Have no colored oxidation products
47	Phenylhydrazonaphthalene	Produces colored oxidation products
48	Diaminodiphenetole	Has no colored oxidation products
49	<i>o</i> - and <i>m</i> -Tolidines	Have no colored oxidation products
50	Tetrabromo- <i>m</i> -tolidine	Has no colored oxidation products
51	<i>o</i> -Tolylphenylamine	Produces colored oxidation products of too little merit to warrant its use
52	Sodium <i>m</i> -tolylphenylaminesulfonate	Has no indicator properties
53	Sodium <i>p</i> -tolylphenylaminesulfonate	Has no indicator properties

in the solution; this acts as the indicator. The results agree with potentiometric titrations.

E. COMPOUNDS REPORTED AS BEING UNSATISFACTORY FOR
USE AS OXIDATION-REDUCTION INDICATORS

In table 1 each compound which has been reported as being unsatisfactory for use as an oxidation-reduction indicator is listed and the reason for its unsuitability is given. In general, the indicators are arranged in alphabetical order in each of the following groups: (1) the diphenylamine series; (2) the complex ions; (3) the commercial dyes; (4) miscellaneous compounds.

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ALKALOIDS OF TOBACCO

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I. INTRODUCTION

The alkaloid nicotine was named after Jean Nicot, consul from the king of France, who in 1560 sent tobacco seed from Portugal to Paris (266).

Nicotine, observed by Vauquelin (248) in 1809 and isolated twenty years later by Posselt and Reimann (188), was considered for many years the only alkaloid in tobacco. Since then other alkaloids,—e.g., anabasine, anatabine, isonicotine, *l*-*N*-methylanabasine, *l*-*N*-methylanatabine, nicotelline, nicotimine, nicotine, nicotyrine, and nornicotine,—have been discovered and isolated from tobacco and other plants. The proportion of nicotine, nicotimine, and nicotelline in tobacco, as compared with 1000 parts of nicotine, is 20, 5, and 1, respectively (177).

Henry (86) stated that members of this series of alkaloids, with one exception, have been found only in *Nicotiana* species combined with citric, malic, oxalic, and other organic acids, the one exception being the Australian plant *Duboisia hopwoodii*, from which nicotine was isolated by Petit (169) and Rothera (201). However, in 1939 Marion (134) extracted a basic oil (0.001 per cent) from the root of the milkweed, *Asclepias syriaca* L.; the oil, boiling at 85°C. at 1 mm., was characterized as *l*-nicotine by the formation of a dipicrate and a dipicolonate which were identical with the corresponding derivatives of an authentic specimen of nicotine.

II. NICOTINE

A. OCCURRENCE

Nicotine has been isolated from *Nicotiana affinis* (257), *N. americana* (213), *N. augustifolia* (248, 257), *N. attenuata* (46), *N. chinensis* (59, 257), *N. glauca* (99), *N. glutinosa* (114, 188, 257), *N. macrophylla* (59, 114, 188, 257), *N. paniculata* (257), *N. persica* (22), *N. rusbyi* (112), *N. rustica* (22, 59, 99, 114, 140, 155, 189, 236, 237, 238, 257), *N. suaveolens* (170), *N. sylvestris* (112), and *N. tabacum* (22, 35, 59, 114, 140, 149, 155, 177, 188, 200, 236, 237, 257, 264).

Bernardini (13) and Chaze (35, 38) were unable to detect nicotine in the seed of the tobacco plant, and Anderson (3) concluded that, at most, the mature seed contained only traces of the alkaloid. Nicotine was found in the sprouts and cotyledons of tobacco seed after only 9 to 11 days of germination (3, 250). Rosenthaler (200), in a diurnal study of the influence of light and aging on the nicotine content of the middle fresh leaves of *N. tabacum*, immediately after picking, found that an increase of nicotine took place from morning to night, while a decrease took place from night to morning. The increase is apparently greater than the decrease, since an accumulation of nicotine finally obtains over an extended period. Il'in (95) observed that, in the germination of tobacco seed during 15 days, both in the light and in the dark, nicotine was absent at first, but that after 5 days it was present in considerable and increasing quantities. Chaze (35, 38) showed the presence of nicotine in the interior of the vacuoles of plants only 1 mm. long and observed that, in the course of the development of the plant, the alkaloid appears throughout the aerial portion. It was found in the interior of the vacuoles which arise from the hydration of the aleurone granules when these often still contained crystalloid protein.

Nicotine is distributed throughout the tobacco plant, the amounts being greatest in the lower leaves and gradually growing less with the age of the leaves; the top leaves contain the least alkaloid (144). Cicerone and Marocchi (42) determined the percentage of nicotine to be greatest in the medium zone of the long axis of the leaf; the marginal zone is richer than the central zone, and in the rib the nicotine content decreases from the apex to the base. Kissling (107) and Il'in (97) found that the nicotine content of the tobacco plant increased with the age of the plant; at the time the young plants were bedded out the nicotine content was 0.15 per cent; after the leaves were formed, 0.20 per cent; before full growth, 0.36 per cent; just after full growth, 0.45 per cent; after gathering, 0.54 per cent; after fermentation, 0.60 per cent. Nishiyama (148) found that the nicotine content increases from the lower to the higher leaves. Koeing (110) found that the nicotine content is highest in the higher leaves, shortly before ripening, and lower in greenhouse, close-planted, and irrigated plants than in normal, open-air plants. Vladescu (251) found that nicotine reaches a maximum at the time of flowering (about 45 days), then decreases about 20 per cent, and finally, at the time of the appearance of the lateral buds, rises again to a higher maximum than before and again decreases. The second maximum is also shown to a lesser extent in plants whose buds are removed from time to time.

Pannain (165), using *Xanthi yoka* grown in Abruzzi, found that before flowering the aerial leaves contained less nicotine than the lower leaves. The leaves of the first and second pickings contained double the quantity

of nicotine present in the leaves of the third and fourth pickings. The leaf blades were always richer in nicotine than the ribs. The stems and roots contained less nicotine than other parts of the plant, but the roots contained more than the stems.

In plants sown very early the nicotine content of the leaves increases from below upward; in late plants the opposite condition prevails; in plants of normal cultivation there is an increase of nicotine in the lower leaves toward the middle leaves, and a decrease in the new leaves toward the plant top (4). Topping causes nicotine to migrate into the rest of the plant (39). The amount of nicotine in the stems can be increased 77 per cent by topping and removing the buds and leaves, then fertilizing with sodium nitrate, and gathering and immediately extracting the stems after the first frost (40).

Chaze (37) concluded that nicotine was a waste product of cellular metabolism and, through morphological and microchemical studies of cells, confirmed the idea that alkaloids are waste products (36), whereas Rosenthaler (200) concluded that tobacco alkaloids cannot, in the strict sense of the word, be regarded as excretion products. Theron and Cutler (238) thought nicotine to be present in the plant as a storage product and not as a protective agent. Il'in (96) considered nicotine as a reserve form of nitrogen participating in the general cycle of biological processes of the organism, its physiological function being analogous to that of asparagine.

B. EXTRACTION

Nicotine is isolated from tobacco commercially by treating the tobacco with an aqueous solution of an alkali and steam distilling. Numerous patents have been issued in this and other countries to individuals and corporations for this purpose, the one obtained by Hyatt (94) on May 17, 1938, for the commercial extraction of nicotine from tobacco, being very satisfactory. Tobacco material (such as stocks, stems, and low-grade leaves) is mixed with an alkali, and the saturated mixture is heated at atmospheric pressure to cause the collapse of the nicotine cells and the vaporization of the liquid present. The pressure on the heated mixture is reduced to effect the expansion and rupture of the collapsed cells. The vapors are condensed under the reduced pressure, and the pressure on the residue is alternately restored and reduced, the vapors being condensed from the residue after each reduction in pressure. (Each restoration of pressure is effected by breaking the vacuum with steam as suddenly as possible to cause the collapse of unexploded cells in the residue, and each reduction in pressure serves to expand and explode the cells so collapsed.)

C. BURNING

In the burning of tobacco, there is formed at the zone of glowing a mixture mainly of nitrogen carbon dioxide.

carbon monoxide, and unused oxygen. The vapors are chiefly of substances already present in the tobacco which have been vaporized at the zone of glowing, such as resins, resin acids, higher hydrocarbons, and nicotine. On cooling, these vapors condense and, as a consequence of the high concentration, form a disperse phase in the form of a mist or fog. As a disperse system, tobacco smoke is capable of existence only up to temperatures of about 300°C. Above this temperature the disperse phase goes over to the vapor phase (262). The amount of nicotine passing unchanged into smoke varies with the rate of smoking. When a cigaret was burned in a closed vessel and the smoke was passed through a series of wash bottles containing dilute sulfuric acid, the whole of the smoke was recovered. On glowing without suction, 45 per cent of the total nicotine was recovered from the smoke; with intermittent glowing and suction (normal smoking), 25 per cent was in the main smoke stream and 14 per cent in the secondary stream from the glowing portion. However, with rapid continuous suction the corresponding figures were 80 and 3 per cent, respectively. The stump underwent no enrichment in nicotine; hence with normal smoking more than 50 per cent of the nicotine is decomposed (260). Justin-Mueller (100), on the other hand, found that, during smoking, nicotine passed through the combustion zone and was completely burned up.

D. PREPARATION

Auerbach and Wolffenstein (6) obtained nicotine by the reduction of nicotine-*N*-oxide with sulfurous or nitrous acids, while Pinner (183) and Pinner and Wolffenstein (186) obtained it by treating oxynicotine with barium hydroxide for 8 to 10 hr. at 140°C. Auzies (9) made nicotine by passing a mixture of pyrrole, ammonia, and butadiene over a mixture of aluminum and thorium oxides and iron, nickel, and cobalt at 350°C. Upon distilling the double chloride of zinc and nicotine with soda lime, Laiblin (116) found the liquid distillate to consist principally of nicotine. Pinner (179) obtained nicotine by reduction of the perbromide of dibromonicotine with zinc dust and hydrochloric acid. Weidel (258) suggested that nicotine may be formed by the action of ammonia on the aldehyde of pyrotartaric acid. Laiblin (117) obtained nicotine when he treated bromonicotine, $C_{10}H_{12}Br_2N_2 + HBr$, with potash. Chattaway and Parkes (33) found that, when nicotine tetrachloriodide is treated with an aqueous solution of sodium hydroxide or sodium sulfite, it decomposes, forming nicotine. Parenty and Grasset (166) obtained nicotine by heating nicotine tetraoxalate to 250°C. If the additive compound of benzoyl chloride and nicotine is heated with strong hydrochloric acid at 100°C., nicotine is formed (182). Späth and Zajic (233) obtained nicotine by treating *l*-nornicotine with formic acid and formaldehyde.

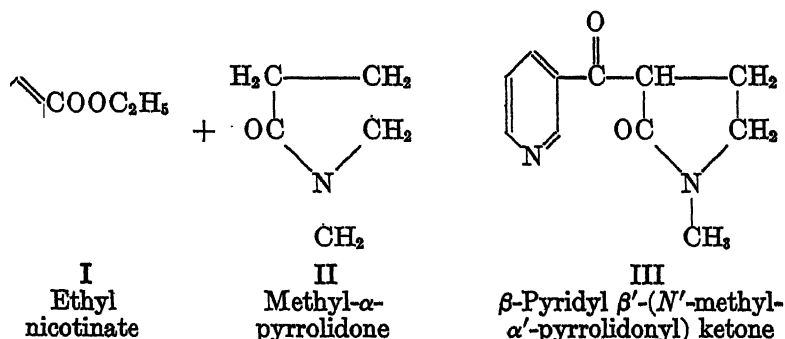
Yamafuji (272) found that the glucoside *tabacilin* is hydrolyzed to glucose, nicotine, and other substances. Barbieri (10) obtained nicotine by treating the tobacco glucoside *tabacin* with strong alkalies; hot concentrated potassium hydroxide converts *tabacol* into nicotine with the liberation of ammonia.

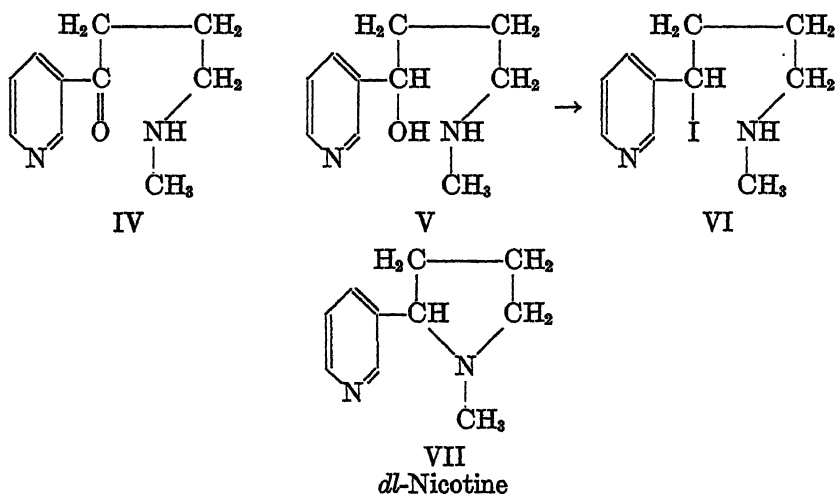
Nicotine may be obtained by steam distilling tobacco in a basic medium, dissolving in a slight excess of hydrochloric acid, recrystallizing several times, and treating with sodium nitrite at a low temperature, the alkaloid being subsequently liberated by alkali, dehydrated, and distilled under reduced pressure (2).

E. SYNTHESIS

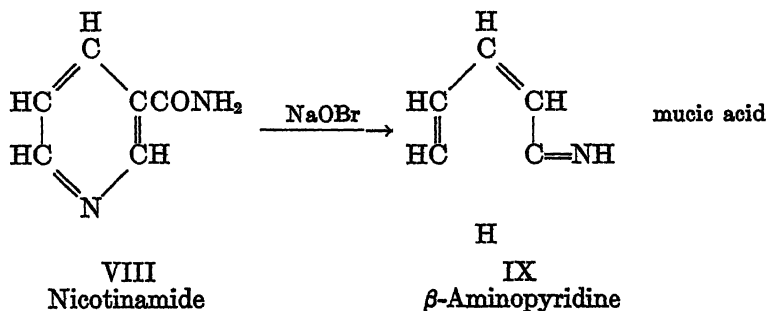
Auzies (8) obtained French patent 425,370 (January 3, 1911) for a synthesis of nicotine, which was effected in four steps: (i) ammonia was conducted over butadiene in the presence of thorium, nickel, aluminum, or copper oxides, with the production of pyrrole; (ii) the pyrrole, after methylating, was hydrogenated in the presence of finely divided nickel; (iii) a mixture of pyrrole and chloroform was heated to 450°C. in the presence of thorium, aluminum, etc., to obtain β -chloropyridine; (iv) nicotine was obtained by heating a mixture of β -chloropyridine and methyl-*l*-pyrrolidine to 425–450°C. in the presence of thorium, aluminum, etc.

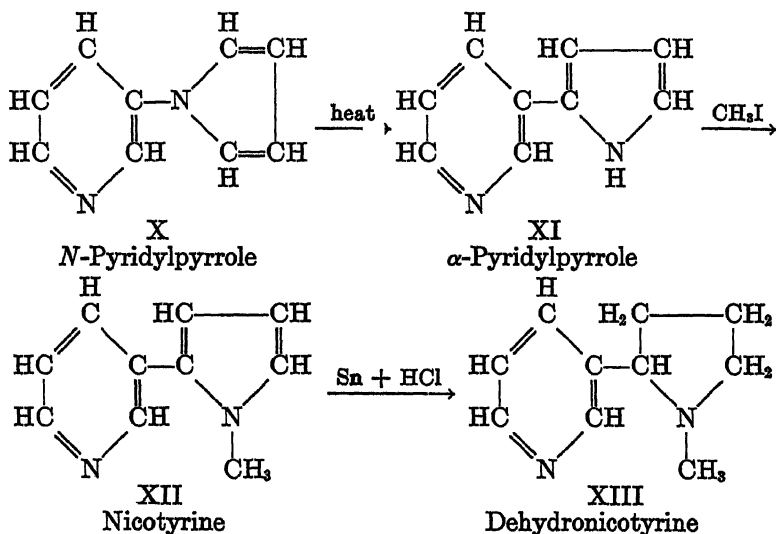
Späth and Bretschneider (223) synthesized nicotine by treating the ethyl ester of nicotinic acid (I) and methyl- α -pyrrolidone (II) with alcohol-free sodium ethylate (giving 70 per cent of β -pyridyl β' -(*N'*-methyl- α' -pyrrolidonyl) ketone (III)). When this ketone was heated for 7 hr. with fuming hydriodic acid at 130°C., the pyrrolidine ring was opened, carbon dioxide was split off, and there was obtained the ketone IV, which was reduced to the alcohol (V) by means of zinc dust in boiling alcoholic alkali. The alcohol, treated with fuming hydriodic acid at 100°C., yielded the iodide VI. The solution of the iodide was made alkaline, was allowed to stand, and was then distilled with steam. The yield of nicotine (VII), identical with racemized nicotine, was 33.4 per cent.



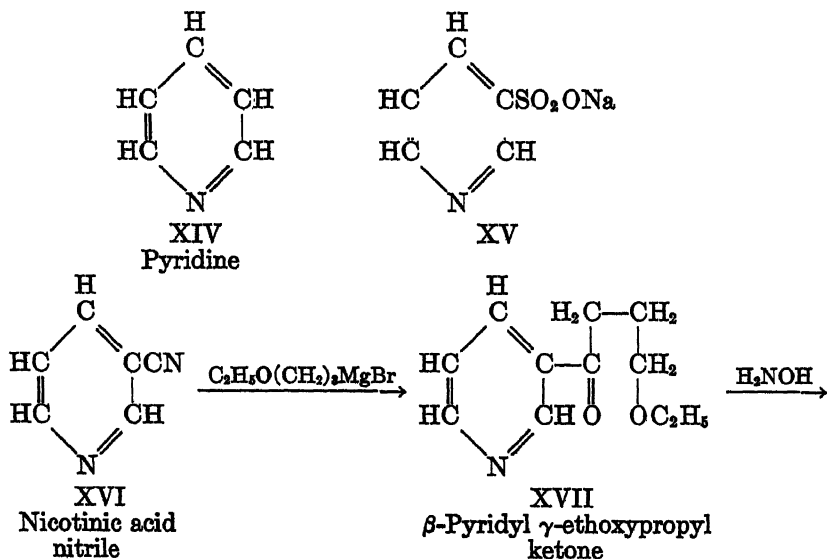


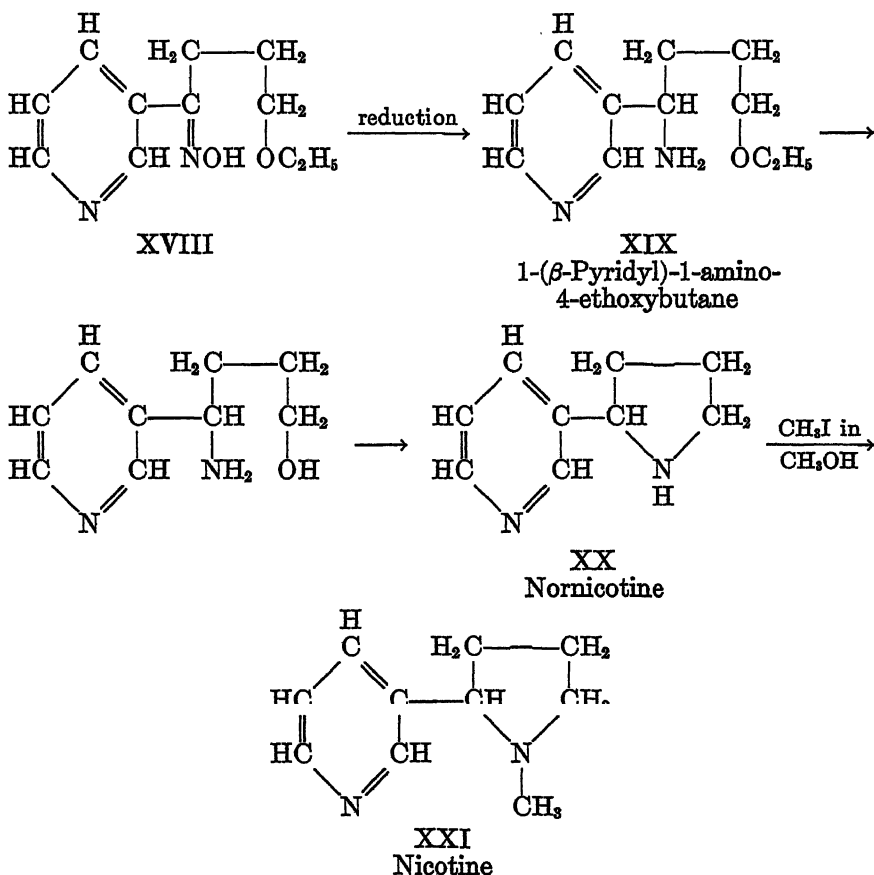
Pictet and Rotschy (178), starting from nicotinic acid, effected the synthesis of nicotine as follows: the amide of nicotinic acid (VIII) was converted, upon treatment with sodium hypobromite, into β -aminopyridine (IX), which, when subjected to dry distillation with mucic acid, formed a pyrrole ring with loss of carbon dioxide and water, giving *N*-pyridylpyrrole (X). When the vapor of this compound was passed through a gently heated ignition tube, molecular rearrangement took place with the formation of the isomeric α -pyridylpyrrole (XI); this substance forms salt-like compounds with alkali metals. Methyl iodide, acting upon the potassium salt, formed nicotyrine (XII). For the purpose of rendering the pyrrole ring of nicotyrine more easily reducible for conversion into nicotine, nicotyrine was converted into a monosubstitution product by means of iodine in alkaline solution. This compound was then reduced to dehydronicotyrine (XIII) by tin and hydrochloric acid. The dibromo addition product of this compound upon reduction with tin and hydrochloric acid gave tetrahydronicotyrine, or inactive nicotine; this racemic compound was converted into its salt with dextrotartaric acid and was thus resolved into *l*-nicotine.





Craig (47) sulfonated pyridine (XIV) with sulfuric acid and then converted the sodium salt (XV) of the product into nicotinic acid nitrile (XVI). The nitrile, when treated with the appropriate Grignard reagent, was converted into β -pyridyl γ -ethoxypropyl ketone (XVII). The ketone formed an oily oxime (XVIII), which upon reduction yielded 1-(β -pyridyl)-1-amino-4-ethoxybutane (XIX). This substance, when deethylated and treated with hydrobromic acid, gave nornicotine (XX) which, upon treatment with methyl iodide in methyl alcohol, formed nicotine (XXI).





F. PHYSICAL PROPERTIES

1. General characteristics

Nicotine is a water-white liquid of sharp burning taste and almost without odor in the cold (131). It is heavier than water and basic in reaction. Its vapor burns in air. It is miscible with water, alcohol, and ether (188). When kept in bottles filled to the stopper and away from light, nicotine remains colorless, only the slightest yellow tint being noticed after 6 months and no change in rotatory power (131). It evaporates appreciably when exposed to air at room temperatures; most of it volatilizes, but part of it turns into a dark resin. Nicotine is volatile in the vapors of ether, light petroleum, or 95 per cent alcohol (145).

2. Boiling point

The following values have been given for the boiling point of nicotine: 109°C. at 20 mm. (131); 115–117°C. at 12 mm. (212, 273); 120°C. at 14

mm. (109); 124–125°C. at 18 mm. (25); 125–140°C. at 16 mm. (265); 240–242°C. (116); 242°C. (175); 243°C. (234); 244°C. (25, 60); 245°C. (123); 246°C. (47, 81, 90, 188, 194, 198); 246.0–246.5°C. at 734.5 mm. (178); 246.1–246.2°C. at 730.5 mm. (171); 246.2°C. at 719.8 mm. (197); 246.6–246.8°C. at 745 mm. (118); 246.7°C. at 745 mm. (2, 26, 62); 247°C. (276); 248°C. (208); 249–250°C. (33); approximately 250°C. (12); 253°C. (183); 266–267°C. (uncorrected) (176).

3. Freezing point

Posselt and Reimann (188) found nicotine to be liquid at $-6^{\circ}\text{C}.$, and Barral (12) found it to be liquid at $-10^{\circ}\text{C}.$ Kruyt (113) was unsuccessful in an attempt to crystallize nicotine at $-50^{\circ}\text{C}.$ Busbey and McIndoo (26) were likewise unable to solidify it at the same temperature, while Pictet and Rotschy (176) were unsuccessful in solidifying nicotine at $-79^{\circ}\text{C}.$ Tsakalotos (242), attempting to crystallize nicotine by freezing, obtained only an amorphous glass, and the freezing-point curve, which would throw light on the existence of the hydrate, could not be realized experimentally.

4. Density

The following values have been reported for the density of nicotine at the temperatures specified: $-80^{\circ}\text{C}.$, 1.095; $-60^{\circ}\text{C}.$, 1.0775 (142): $-49.31^{\circ}\text{C}.$, 1.0627 (212); $-40^{\circ}\text{C}.$, 1.06 (142); $-39.55^{\circ}\text{C}.$, 1.0546; $-28.95^{\circ}\text{C}.$, 1.0465 (212); $-20^{\circ}\text{C}.$, 1.043 (142); $-19.09^{\circ}\text{C}.$, 1.0392; $-9.19^{\circ}\text{C}.$, 1.0305 (212); $0^{\circ}\text{C}.$, 1.027 (142); 1.0258 (19); $0.75^{\circ}\text{C}.$, 1.023 (212); $10^{\circ}\text{C}.$, 1.0175 (19); $11.41^{\circ}\text{C}.$, 1.0147 (212); $15.6^{\circ}\text{C}.$, 1.012 (89); $20^{\circ}\text{C}.$, 1.0107 (142); 1.01022, 1.0101 (129); 1.0096 (19); 1.00925 (98); $20.55^{\circ}\text{C}.$, 1.0072 (212); $30^{\circ}\text{C}.$, 1.0014 (19); 1.00124 (203); $30.19^{\circ}\text{C}.$, 1.0000; $39.83^{\circ}\text{C}.$, 0.9915 (212); $40^{\circ}\text{C}.$, 0.99383 (203); 0.9935 (19); $50^{\circ}\text{C}.$, 0.9863 (203); $51.01^{\circ}\text{C}.$, 0.9837 (212); $60^{\circ}\text{C}.$, 0.9789 (203); $60.66^{\circ}\text{C}.$, 0.976; $70.86^{\circ}\text{C}.$, 0.9665; $79.06^{\circ}\text{C}.$, 0.9607; $89.07^{\circ}\text{C}.$, 0.9527; $93.45^{\circ}\text{C}.$, 0.949 (212).

5. Specific gravity

Values found in the literature for the specific gravity of nicotine are given below: $d_4^{0^{\circ}}$, 1.0212 (168); $d_4^{4^{\circ}}$, 1.033 (12, 215); $d_4^{10^{\circ}}$, 1.0177 (178); $d_4^{10.2^{\circ}}$, 1.01837 (118); $d_4^{12.5^{\circ}}$, 1.0778 (176); $d_4^{14.2^{\circ}}$, 1.0108 (168); $d_4^{14.8^{\circ}}$, 1.0124 (168); $d_4^{16^{\circ}}$, 1.027 (12), 1.013 (123), 1.0111 (215); $d_4^{17.1^{\circ}}$, 1.0119 (268); $d_4^{18.3^{\circ}}$, 1.0117 (25); $d_4^{19.27^{\circ}}$, 1.0081 (212); $d_4^{19.4^{\circ}}$, 1.0095 (175); $d_4^{20^{\circ}}$, 1.0145 (25), 1.01122 (129), 1.01101 (118), 1.011 (211), 1.01071 (69), 1.01049 (85), 1.01002 (129), 1.01 (168), 1.00995 (268), 1.00984 (129), 1.0098 (15), 1.0097 (178), 1.0096 (235), 1.0095 (168, 194), 1.00947 (197), 1.00931 (131), 1.0093 (26), 1.00925 and 1.00924 (197), 1.0092 (98, 178, 197), 1.0091 (242, 243), 1.009

(171); $d_{40}^{21.1^\circ}$, 1.00865 (98); $d_{40}^{22.4^\circ}$, 1.027 (142), 1.0212 (168), 1.0121 (25), 1.008 (53); $d_{40}^{26.1^\circ}$, 1.0064 (87); $d_{40}^{29.5^\circ}$, 1.0017 (98); $d_{40}^{30^\circ}$, 1.018 (12), 1.00373 (118), 0.99567 (203); $d_{40}^{30.18^\circ}$, 1.0001 (212); $d_{40}^{39^\circ}$, 0.9925 (168); $d_{40}^{40^\circ}$, 0.99424 (98), 0.99225 (203); $d_{40}^{41^\circ}$, 0.99037 (168); $d_{40}^{41.5^\circ}$, 0.9924 (98); $d_{40}^{41.6^\circ}$, 0.99334; $d_{40}^{42.7^\circ}$, 0.9926 (268); $d_{40}^{43.49^\circ}$, 0.985 (212); $d_{40}^{50^\circ}$, 1.0006 (12), 0.98807 (203); $d_{40}^{52^\circ}$, 0.984 (98); $d_{40}^{59.98^\circ}$, 0.9765 (212); $d_{40}^{60^\circ}$, 0.98324 (203), 0.97799 (98); $d_{40}^{61.6^\circ}$, 0.9777 (268); $d_{40}^{62^\circ}$, 0.976 (98); $d_{40}^{63.7^\circ}$, 0.9755 (268); $d_{40}^{69.6^\circ}$, 0.9699 (98); $d_{40}^{70^\circ}$, 0.9688; $d_{40}^{71.5^\circ}$, 0.9673 (168); $d_{40}^{73.3^\circ}$, 0.9685 (87); $d_{40}^{74^\circ}$, 0.96797 (87); $d_{40}^{76.98^\circ}$, 0.9631 (212); $d_{40}^{80^\circ}$, 0.96184 (98); $d_{40}^{82^\circ}$, 0.96081 (268); $d_{40}^{82.1^\circ}$, 0.9607 (268); $d_{40}^{86.4^\circ}$, 0.9567 (98); $d_{40}^{89.4^\circ}$, 0.9552 (87); $d_{40}^{92^\circ}$, 0.9521 (98); $d_{40}^{97.64^\circ}$, 0.9465 (212); $d_{40}^{97.7^\circ}$, 0.94534 (98); $d_{40}^{99^\circ}$, 0.947 (268), 0.9453; $d_{40}^{99.2^\circ}$, 0.9449 (168); $d_{40}^{99.5^\circ}$, 0.94622 (268); $d_{40}^{101.5^\circ}$, 0.9424 (12); $d_{40}^{113.22^\circ}$, 0.9297 (212); $d_{40}^{121^\circ}$, 0.92794; $d_{40}^{121.3^\circ}$, 0.9277 (268); $d_{40}^{133.91^\circ}$, 0.9131; $d_{40}^{163.92^\circ}$, 0.8923; $d_{40}^{183.18^\circ}$, 0.8763; $d_{40}^{203.28^\circ}$, 0.8495 (212).

6. Miscibility with solvents

(a) *With water.* Hudson (93) observed that nicotine and water are miscible in all proportions at temperatures below 60°C. and above 210°C. At temperatures between 60°C. and 210°C. the miscibility is limited, except when one of the components is present in very large excess. Above 90°C., a saturated solution of water in nicotine is lighter than a saturated solution of nicotine in water; below 90°C., the reverse is the case. When nicotine is mixed with water, much heat is developed, probably owing to the formation of a hydrate (192). The marked variation of the specific rotation and of the refractive index with the concentration points to the same interpretation. To the presence of such a hydrate the miscibility of the two otherwise immiscible liquids, nicotine and water, may be attributed.

The data of table 1 are graphically presented in figure 1.

Jephcott (98) found that, owing to the so-called closed curve of the solubility of nicotine in water, it is not possible to observe the rotatory power and density of solutions containing between 7 and 87 per cent of nicotine at all temperatures up to 100°C., since separation occurs at about 60°C. Two solutions were therefore prepared which would fall outside this closed curve and which contained 6.638 per cent and 88.338 per cent of nicotine. From these solutions the data in table 2 were obtained.

The formation of hydrates of nicotine and their decomposition at higher temperatures show the true nature of the closed curve of solubility. Nicotine is only sparingly soluble in water, and water is only sparingly soluble in nicotine, but hydrates of nicotine are miscible with water, a state of balance existing at any given temperature between nicotine, its hydrates, and water.

TABLE 1
Solutions of nicotine in water

NICOTINE	TEMPERATURE	
	Decomposition	Remixing
<i>per cent</i>	$^{\circ}\text{C.}$	$^{\circ}\text{C.}$
6.8	94	95
7.8	89	155
10.0	75	
14.8	65	200
32.2	61	210
49.0	64	205
66.8	72	190
80.2	87	170
82.0	129	130

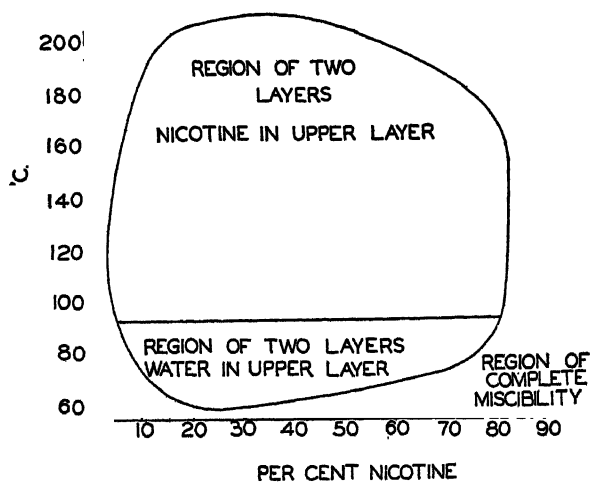


FIG. 1. Miscibility of nicotine and water

TABLE 2
Solutions of nicotine in water

TEMPERATURE	NICOTINE		d_{4}^{t}	$[\alpha]_{D}^{t}$
$^{\circ}\text{C.}$	<i>per cent by weight</i>	<i>grams in 100 cc. of solution</i>		
20	6.638	6.6820	1.00275	-76.82°
85	6.638	6.4188	0.96328	-95.29
20	88.338	90.8200	1.02810	-134.16
90	88.338	86.9360	0.98412	-150.34

As the temperature rises, the hydrate formation reverses, and when the concentration of free nicotine becomes greater than the solubility of nicotine in water at that temperature, separation occurs. By choosing concentrations of nicotine and water such that the limit of solubility of the one in the other is not exceeded, it is possible to note the marked rise in rotatory power as the concentration of free nicotine increases with rise in temperature (242).

Measurements of the viscosity of mixtures of water and nicotine show that there is an exceptionally great increase in viscosity on mixing. The index of refraction for the mixtures shows a straight line. The author considers that the peculiar behavior of this binary system is due to the formation of a hydrate of nicotine.

TABLE 3
Reciprocal solubility of the system nicotine-water

NICOTINE	WATER	TEMPERATURE OF SATURATION	
		Lower	Higher
<i>per cent</i>	<i>per cent</i>	<i>°C.</i>	<i>°C.</i>
5.4	94.6	Homogeneous	
6.5	93.5	92.7	94.5
15.3	84.7	64.3	195.0
33.4	66.6	60.8	208.0
49.6	50.4	62.7	204.0
66.7	33.3	71.8	194.0
71.7	28.3	75.8	185.0
79.2	20.8	86.1	168.0
84.5	15.6	Homogeneous	

According to Seyer and Gallagher (212), nicotine dissolves in its own volume of water to form a sticky, viscous liquid, which, on warming, becomes turbid and separates into two liquid layers, the upper being a saturated solution of nicotine in water, and the lower a saturated solution of water in nicotine. Further warming will again bring about the formation of a single homogeneous phase. Ever since the discovery of the ring-shaped solubility curve of nicotine and water (93), attempts have been made to show the existence of hydrates at the temperatures below the critical (lower) temperature.

Considerable work has been done with the object of establishing the existence of these hydrates. Owing to the viscous nature of the solutions at temperatures below 20°C., it has so far been difficult to obtain reliable freezing-point data and quite impossible to separate any hydrates which might have been formed. While Jephcott (98), upon an examination of

the freezing points of solutions of nicotine, found indications of hydrates, still, most of the evidence supporting the theory of hydrate formation has come from the investigation of other physical properties of nicotine and water solutions. Thus Tsakalotos (244) investigated the viscosity, Skalweit (215) the specific gravity, and Winther (268) the index of refraction and specific rotatory power, all with a view toward offering an explanation of the closed solubility curve.

The system water-nicotine is one of the few known examples of a system in which there are two critical temperatures of solution, i.e., in which the temperature-concentration curve is a closed curve, two strata existing only within a definite interval of temperature above or below which only a single homogeneous phase exists (93). Redetermination of data for the system water-nicotine by Leone (120) confirmed the data of Hudson (93), except for very small variations. Acetone, which is miscible with each component in all proportions, was then introduced as a third component. The data showed that acetone increases the mutual solubility of water and nicotine, reducing the temperature zone of existence of the two phases until, with enough acetone, the two phases disappear. The maximum distance between the two critical points occurs with the mixture containing 31.73 per cent of nicotine, and the maximum concentration of acetone which still allows the existence of a non-miscibility zone is 35.44 per cent, at which concentration this zone is so greatly reduced that it presents only a slight opalescence between 146° and 155°C. With a slightly greater concentration of acetone, two phases are not formed on heating. The molecular variations of the critical temperature calculated by Timmerman (240) are not constant for all proportions of water and nicotine, but tend to increase slightly with increase in the concentration of acetone. In the critical zone between 20 and 40 per cent nicotine, the general relation between the ratios of the molecular variations is constant. The molecular variations of acetone remain within values of 100 to 150 for the lower critical temperatures while, for the higher critical temperatures, they hardly reach 100. For a given sample, the molecular variation in the lower critical temperature is 30–40°C. greater than the corresponding variation in the higher critical temperature. A distinct and constant difference between the values of the molecular increases of the lower critical temperature and the corresponding molecular lowering of the higher critical temperature supports the hypothesis that the two critical points must be interpreted differently.

Nicotine is miscible with most liquids at the ordinary temperature, and the radius of its molecular sphere should approximate that of pyridine (87). The values given in table 4 are calculated for water-nicotine mixtures at 20°C. (193).

It is evident that these results differ materially from any obtained before, inasmuch as the greatest difference between the percentage of nicotine in the initial and the final volumes occurs at a mixture well removed from equimolecular proportions referred to the gaseous state. From the data

TABLE 4
Equimolecular volumes of gaseous nicotine
 $100\text{C}_{10}\text{H}_{14}\text{N}_2:11.24\text{H}_2\text{O}$

MOLECULAR MIXTURE	CHANGE IN VOLUME*	Δ nicotine†
Nicotine-water.....	-1.88	1.545
Nicotine-1.5 water.....	-2.62	1.963
Nicotine-2 water.....	-3.20	2.190
Nicotine-2.5 water.....	-3.70	2.322
Nicotine-3 water.....	-4.17	2.407
Nicotine-3.5 water.....	-4.45	2.368
Nicotine-4 water.....	-4.75	2.338

* Change in volume when the volume of the particular liquid in question is constant at 100 volumes, the signs (+) and (-) referring to expansion and contraction, respectively.

† Differences (Δ) between the proportion by volume of this liquid in the initial and final volumes.

TABLE 5
Equimolecular volumes of gaseous nicotine
 $100\text{C}_{10}\text{H}_{14}\text{N}_2:25.14\text{CH}_2\text{OH}$

MOLECULAR MIXTURE	CHANGE IN VOLUME*	Δ nicotine†
Nicotine-alcohol.....	-1.15	0.742
Nicotine-1.5 alcohol.....	-1.58	0.843
Nicotine-2 alcohol.....	-1.93	0.966
Nicotine-2.5 alcohol.....	-2.20	0.841
Nicotine-3 alcohol.....	-2.45	0.808

* Change in volume when the volume of the particular liquid in question is constant at 100 volumes, the signs (+) and (-) referring to expansion and contraction, respectively.

† Differences (Δ) between the proportion by volume of this liquid in the initial and final volumes.

in table 4 the complexity of nicotine would appear to be one-half that of water, and the equimolecular liquid mixture would be $\text{C}_{10}\text{H}_{14}\text{N}_2 \cdot 2\text{H}_2\text{O}$.

A similar complexity for nicotine is deducible from its mixtures with methyl alcohol at 20°C. (268) (see table 5). Assuming similar complexity, the ratio of the radius of the nicotine molecule to that of water as unity at 20°C. would be

$$\overline{1.00995} \quad \overline{0.99823} \quad \text{or } 2.07$$

A mixture was prepared containing 1 mole of nicotine to 2 moles of water in the liquid state, and the temperature at which partial miscibility commenced was determined. Heated in a sealed tube, the mixture remained homogeneous until a temperature of 75.8°C. was reached, when a cloudiness appeared at the surface; at 76°C. this cloudiness had spread through the mass of the liquid and two layers separated.

The initial volumes of nicotine and water in the mixture at 74°C. are 21.162 cc. and 9.333 cc., respectively, and the final volume is 29.898 cc.; i.e., 100 volumes of nicotine when mixed with 44.10 volumes of water contracted to the extent of 2.82 volumes, so that at this temperature the volume change is still considerable.

The ratio of the radii of the spheres of nicotine and water increases with rise in temperature and at 26.1°, 73.3°, and 89.8°C. is 1.64, 1.65, and 1.66, respectively. These values are so close to that required theoretically for partial miscibility that they may be deemed sufficient confirmation of the complexity found above for nicotine: namely, one-half that of water.

The following table gives the radial ratios of the molecular spheres of the liquids, which, on admixture at the stated critical temperatures of solution, are on the immediate borderline between complete and partial miscibility:

MIXTURE	CRITICAL TEMPERATURE OF SOLUTION OF THE MOLECULAR MIXTURE	RATIO OF MOLECULAR RADII AT THE CRITICAL TEMPERATURE
Nicotine and water.....	76°C.	1.65

It will be noticed that the ratio, 1.65, is greater than the theoretical value, 1.62. Nicotine and water give the nearest approach to this value, and they also give the greatest contraction in volume on admixture.

(b) *With petroleum.* A sample of American petroleum was purified from unsaturated and aromatic compounds and fractionated repeatedly so as to remove the fractions of lower boiling points. The fraction boiling between 200° and 230°C. was taken for a study of petroleum-nicotine-water mixtures (88).

(1) The initial proportions by volume of the constituents at 15°C. were as follows: petroleum, 33.3; water, 33.3; nicotine, 33.3. After vigorous shaking at 5°C., this mixture of liquids separated into two layers having the relative proportions: petroleum layer, 36; water-nicotine layer, 64. When the mixture was heated to 62°C., the lower aqueous layer became cloudy and separated rapidly into two layers, a lower layer containing

nicotine and an intermediate aqueous layer which increased in volume as the temperature rose until, at 90°C., the relative proportions were as follows: petroleum layer, 41; water layer, 26; nicotine layer, 33. On mixing at the latter temperature, two layers were again formed, owing to the nicotine dissolving in the petroleum. The observed proportions of the layers were now: petroleum-nicotine layer, 67; aqueous layer, 33. Both layers remained clear at temperatures above 63°C. At immediately lower temperatures the nicotine began to separate from the petroleum and fell to the bottom of the tube, leaving at first an intermediate aqueous layer, which, however, on further cooling, mixed with the nicotine, and the liquids resumed their original equilibrium of two layers.

(2) The initial proportions by volume of the constituents at 15°C. were as follows: petroleum, 6.7; water, 33.3; nicotine, 60.0. At 5°C. this mixture of liquids formed two layers in the relative proportions: petroleum layer, 8; nicotine layer, 92. When the temperature was raised to 67°C.,

TABLE 6
Molecular complexity of nicotine

LIQUID	TEMPERATURE	COMPLEXITY IN THE LIQUID STATE WATER = (H ₂ O) ₄	MOLECULAR VOLUMES COMPARED WITH THAT OF WATER AS UNITY AT THE SAME TEMPERATURE		MOLECULAR RADIUS AS COMPARED WITH THAT OF WATER
			Referred to the gaseous state	Referred to the liquid state	
Nicotine.....	°C. 20	(C ₁₀ H ₁₄ N ₂) ₂	8.92	4.46	1.65

a cloudiness appeared in the aqueous layer, and three layers were formed which at 90°C. had the following relative proportions: petroleum layer, 8; aqueous layer, 9; nicotine layer, 83. At 100°C., the lower nicotine layer rose to the middle portion and dissolved in the petroleum, so that, on mixing at this temperature, two layers were again formed in the proportions: petroleum-nicotine layer, 75; aqueous layer, 25. On cooling, the nicotine began to separate from the petroleum and fell to the bottom of the tube at 68°C.; its general behavior at lower temperatures was similar to that in the preceding mixture. Very gradual cooling of this mixture resulted in the formation of four layers at some temperatures. These consisted of a small petroleum layer, then a large layer of petroleum and nicotine, and below these an aqueous layer, and then at the bottom a concentrated nicotine layer. These layers, however, were clearly metastable, and on mixing gave two stable layers only.

(3) The initial proportions by volume of the constituents at 15°C. were as follows: petroleum, 60.0; water, 33.3; nicotine, 6.7. At 5°C. the

proportions of the two layers formed were as follows: petroleum layer, 60; water-nicotine layer, 40. The lower aqueous layer became cloudy at 76°C., and then results similar to those obtained with the preceding mixtures were observed.

It will be noticed that at some concentrations the foregoing mixtures of liquids form two layers only, which, within a limited range of temperature, yields three-layer systems, proving how close is the relationship of the molal volumes of the several liquids to the ratio required by theory for partial miscibility.

(c) *With aqueous solutions.* Dubrisay (56) determined the miscibility of nicotine with water and with solutions of sodium hydroxide, hydrochloric acid, and sodium sulfate (see table 7).

TABLE 7

Miscibility of nicotine with water and with solutions of hydrochloric acid, sodium sulfate, and sodium hydroxide

WATER-NICOTINE		N/10 HCl-NICOTINE		N/10 Na ₂ SO ₄ -NICOTINE		N/10 NaOH-NICOTINE	
Concentration	Temperature	Concentration	Temperature	Concentration	Temperature	Concentration	Temperature
per cent	°C.	per cent	°C.	per cent	°C.	per cent	°C.
66.60	70.8	66.6	71.1	40.0	40.5	50.0	51.1
63.33	68.0	50.0	70.2	33.3	47.0	33.3	55.2
55.55	66.8	40.0	70.2	25.0	49.1	25.0	56.0
50.00	63.1	33.3	73.2	20.0	51.9	20.0	57.8
40.00	62.3						
33.33	62.0						
25.00	62.3						
20.00	62.3						

7. Adsorption

The amounts of nicotine adsorbed from 25 cc. of 0.01 *M* solution by 0.5 g. of various adsorbents are as follows: silica, 87.3 per cent; alumina, 1.5 per cent; iron oxide, 2.2 per cent; fuller's earth, 43.7 per cent. The adsorption by hydrous iron oxide was irregular, being 0.2 and 2.7 per cent, respectively, for oxide samples prepared by precipitation from ferric chloride by means of ammonia solution and by the oxidation of electrolytic iron with ozone in the presence of water and platinum foil (77).

8. Volatility

The concentration of nicotine in the vapor phase was determined by Harlan and Hixon (80) at 25°, 30°, 35°, and 40°C. by the "air-bubbling method." These data are reported in table 8.

TABLE 8
Vapor concentrations of nicotine

TEMPERATURE	AIR PASSED OVER NICOTINE	NICOTINE OBTAINED	CONCENTRATION OF NICOTINE	
			mg. per 10 liters of air	p.p.m.
°C.	liters	grams		
25	60	0.01106	1.84	28.5
25	60	0.01056	1.76	27.3
30	60	0.01635	2.73	43.0
30	60	0.01596	2.66	41.9
35	30	0.01250	4.17	66.8
35	30	0.01232	4.11	65.9
40	30	0.01723	5.74	94.3
40	30	0.01812	6.04	99.2

TABLE 9
Viscosity of the system nicotine-water at : °C.

per cent	DURATION OF FLOW seconds
33.4	249.2
49.6	583.0
66.7	1355.0
71.7	1727.5
73.9	1821.0
79.2	1866.0
86.1	1511.0
100.0	247.2

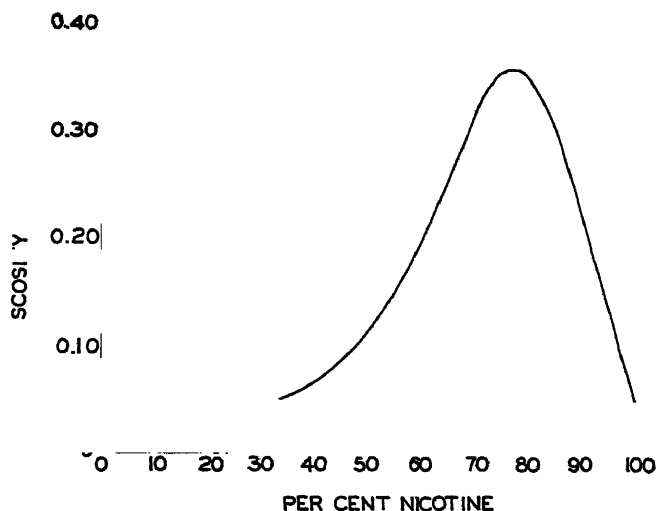


FIG. 2. Viscosity of the system nicotine-water at 20°C.

9. Viscosity

Values for the viscosity of the system nicotine-water at 20°C. are given in table 9. The curve resulting from a plot of these values (figure 2) shows a maximum at the concentration of 78 per cent nicotine and indicates the formation of a molecular combination between water and nicotine. Measurements of the viscosity of the liquids show that there is an exceptionally great increase in viscosity on mixing. The viscosity of the mixture is eight times greater than that of nicotine and thirty-five times that of water. According to Deželić (53), the viscosity curve with pyrrole shows a definite maximum at a concentration of approximately 60 mole per cent of nicotine (see figure 3).

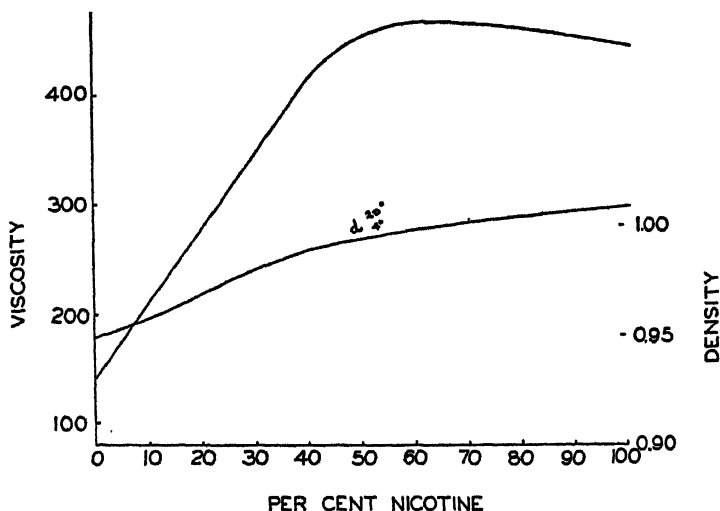


FIG. 3. Viscosity of the system nicotine-pyrrole at 20°C.

The viscosity curve of nicotine and 2,4-dimethylpyrrole possesses a sharply marked maximum which is exactly at 33.3 per cent nicotine (2 moles of 2,4-dimethylpyrrole:1 mole of nicotine); in the system 2,4-dimethyl-3-ethylpyrrole (cryptopyrrole)-nicotine the viscosity curve tapers to a point, a maximum being situated at 32 per cent nicotine (2 moles of cryptopyrrole:1 mole of nicotine) (54) (see figure 4).

10. Vapor pressure

The vapor pressure measurements made by Gorbatshev (75) were carried out by determining the boiling points in a manostat and the saturation pressures within a range of 90–230°C. (see figure 5a). The determination of the vapor pressure by Young and Nelson (273) is by the air saturation

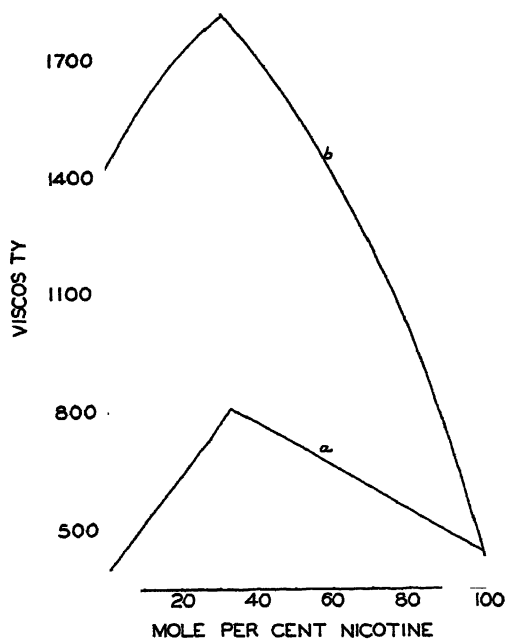


FIG. 4. Viscosity of (a) the system nicotine-2,4-dimethylpyrrole and (b) the system nicotine-2,4-dimethyl-3-ethylpyrrole.

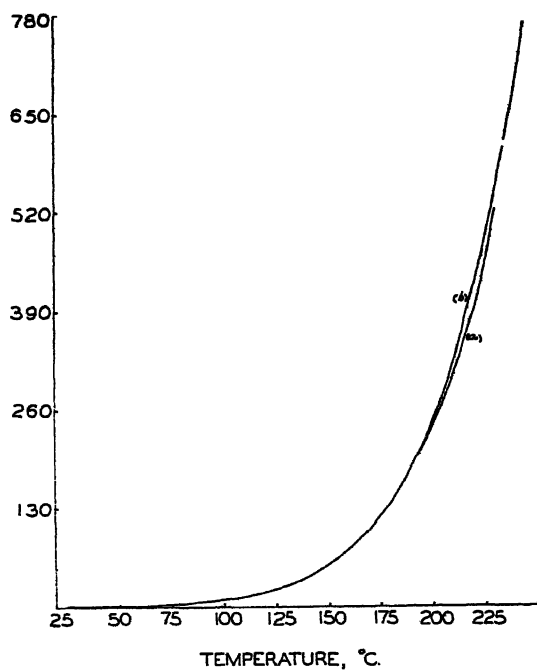


FIG. 5. Vapor pressure of nicotine

method up to 100°C. and by the static method from this point to the boiling point of nicotine (see figure 5b).

Schükarew (208) found the vapor pressure of mixtures of nicotine and water to be constant between 17 and 82 per cent nicotine at the lower critical solution temperature. At the upper critical solution temperature, the vapor pressure of the solution is generally greater than that of the more volatile component.

11. Surface tension

Seyer and Gallagher (212) calculated the surface tension values by means of the formula $\gamma = K Hd$, where γ is the surface tension in dynes, K is a constant for the apparatus employed, H is the corrected difference in height of liquid in the two capillaries, and d is the density of the solutions. The Eötvös constant in the case of nicotine was obtained by plotting the molecular surface energies against temperature on a large scale and then taking the difference in the molecular surface energies from the curves over intervals of about 30°C.

It is futile to draw any conclusions as to the state of nicotine and water in the various solutions from a consideration of the change in molecular surface energy with temperature. Skalweit (215) observed a maximum density at a concentration of 72 per cent nicotine, Tsakalotos (242) a viscosity maximum at 78 per cent, and Jephcott (98) from the specific rotation of nicotine solutions found maxima at concentrations of roughly 40 and 80 per cent nicotine. According to Denison (51), such deviations can scarcely be due to the change in association of either solvent. The most likely explanation of the maxima must be that they represent the points of maximum concentration of nicotine hydrates, which break down as the temperature approaches 60°C.

Semenchenka and Ivanova (209) measured the interfacial surface tension of nicotine by the drop method, using butyric acid, butyraldehyde, butyronitrile, butyramide, propyl alcohol, and propyl chloride at various concentrations and temperatures, as shown in table 11. It was found that all the compounds used had a high surface activity which was directly proportional to the dipole moment for compounds not containing nitrogen and inversely proportional to the dipole moment for compounds containing nitrogen.

12. Density of mixtures of nicotine with other liquids

(a) *Water and nicotine.* Seyer and Gallagher (212) determined the density of six solutions of nicotine and water which contained, per 100 g. of solution, the following quantities of nicotine: (a) 5.35 g.; (b) 10.12 g.; (c) 23.74 g.; (d) 35.15 g.; (e) 54.86 g.; and (f) 72.16 g. From these data the graphs of figure 6 have been drawn. It is noticed that with increasing

TABLE 10
Surface tension and other data of the system nicotine-water

TEMPERATURE	DENSITY	SURFACE TENSION	MOLECULAR SURFACE ENERGY	BOILING CONSTANT K_b
100 per cent nicotine				
$^{\circ}\text{C}.$		<i>dynes per cm.</i>	<i>ergs per sq. cm.</i>	
-49.31	1.0627	46.58	1331	2.5
-39.55	1.0546	45.39	1307	
-28.95	1.0465	44.12	1277	
-19.09	1.0392	43.16	1244	
-9.19	1.0305	42.22	1227	
0.75	1.0230	41.41	1206	2.6
11.41	1.0147	39.89	1174	
20.55	1.0072	38.61	1141	
30.19	1.0000	37.70	1121	2.7
39.83	0.9915	36.54	1092	
51.01	0.9837	35.37	1063	
60.66	0.9760	34.15	1032	2.8
70.86	0.9665	33.08	1005	
79.06	0.9607	32.17	983	
89.07	0.9527	31.25	959	2.4
93.45	0.9490	30.99	953	
Mixture: 5.35 g. of nicotine per 100 g. of solution				
0.09	1.0029	57.13	404.6	1.6
6.20	1.0025	55.25	391.8	
10.30	1.0021	54.39	385.5	
20.80	1.0003	52.14	370.0	2.2
29.70	0.9978	47.91	340.0	
41.50	0.9940	43.65	326.6	
52.10	0.9893	41.85	299.1	1.1
60.53	0.9845	38.94	279.3	
70.30	0.9793	37.11	267.1	
79.97	0.9740	34.84	252.8	1.3
90.27	0.9685	33.54	243.2	
95.40	0.9685	32.30	234.6	
Mixture: 35.15 g. of nicotine per 100 g. of solution				
0.24	1.0314	51.55	444.5	1.4
7.70	1.0284	50.34	435.5	
19.90	1.0223	47.54	412.7	
24.80	1.0195	46.73	407.4	
30.56	1.0157	45.68	399.3	
35.30	1.0130	44.92	392.1	1.8
46.20	1.0055	42.13	365.5	
55.86	0.9982	40.06	353.4	
Mixture: 72.16 g. of nicotine per 100 g. of solution				
0.42	1.0540	47.37	622.3	1.8
5.50	1.0500	46.75	615.2	
10.20	1.0462	45.88	605.7	
19.85	1.0374	44.29	587.5	
25.02	1.0327	43.30	576.8	
29.17	1.0288	42.65	570.6	1.6
34.97	1.0236	41.77	560.4	
40.30	1.0185	40.90	548.8	
50.45	1.0091	39.67	536.5	1.4
60.00	1.0002	38.49	523.6	
69.85	0.9914	37.10	507.6	
75.05	0.9862	36.58	502.3	

nicotine concentration the temperature-density relationships become almost linear.

Table 12 gives values for the density and specific rotation of mixtures of nicotine and water; these data are represented graphically in figure 7.

TABLE 11
Interfacial surface tension of nicotine

	MOLE PER CENT	TEMPERATURE OF SOLUTION	70°C.	80°C.
Butyric acid.....	0.00	60.2	0.304	0.400
	0.14	64.2	0.075	
	0.26	68.0	0.077	
	0.30	68.8	0.061	
	0.44	72.4	0.065	
Butyramide.....	0.19	61.0	0.210	0.335
	0.24	62.2	0.185	0.286
	0.38	63.7	0.163	0.267
	0.60	65.7	0.152	0.269
Butyronitrile.....	0.16	58.9		0.325
	0.37	57.2	0.105	0.366
	0.57	55.5	0.136	0.463
	0.73	54.3	0.207	0.641
Butyraldehyde.....	0.20	55.9		0.337
	0.35	52.2	0.105	0.470
	0.45	50.2	0.166	0.576
	0.60	47.0	0.265	0.790
Propyl alcohol.....	0.00	60.2	0.304	0.400
	0.21	60.4	0.280	0.370
	0.39	60.5	0.263	0.365
	0.61	60.8	0.270	0.365
	0.80	61.2	0.261	0.360
Propyl chloride....	0.20	53.6		0.381
	0.33	48.4	0.167	0.542
	0.49	43.2	0.290	0.782
	0.68	41.8	0.595	1.123

In table 13 are listed additional values for the density of the system nicotine-water, while the data are represented graphically in figure 8. From the same source were derived the data on the coefficient of contraction of nicotine-water mixtures (see table 14 and figure 9). Table 15 also presents data on the density of the system nicotine-water. The

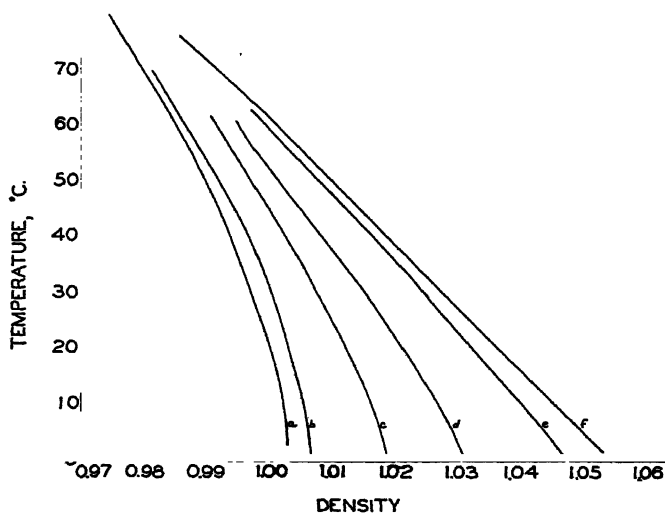


FIG. 6. Density of the system water-nicotine: curve a, 5.35 g. of nicotine per 100 g. of solution; curve b, 10.12 g. of nicotine per 100 g. of solution; curve c, 23.74 g. of nicotine per 100 g. of solution; curve d, 35.15 g. of nicotine per 100 g. of solution; curve e, 54.86 g. of nicotine per 100 g. of solution; curve f, 72.16 g. of nicotine per 100 g. of solution.

TABLE 12
Density and specific rotation of the system nicotine-water (98)

GRAMS OF NICO- TINE PER 100 CC. OF SOLUTION	$d_4^{20^\circ}$	SPECIFIC ROTA- TION $[\alpha]_D^{20^\circ}$	GRAMS OF NICO- TINE PER 100 CC. OF SOLUTION	$d_4^{20^\circ}$	SPECIFIC ROTA- TION $[\alpha]_D^{20^\circ}$
100.925	1.00925	-168.61°	45.296	1.02936	-86.47°
96.801	1.01823	-153.06	42.882	1.02790	-86.71
93.323	1.02458	-141.65	41.308	1.02661	-85.09
91.781	1.02583	-138.73	39.804	1.02592	-83.79
90.820	1.02810	-134.11	39.025	1.02522	-85.21
86.132	1.03356	-123.21	38.950	1.02538	-84.98
84.632	1.03439	-121.48	35.920	1.02431	-83.52
79.921	1.03784	-111.47	35.696	1.02351	-83.39
78.551	1.03836	-108.39	32.810	1.02107	-81.83
77.764	1.03839	-108.69	31.607	1.02048	-82.48
71.963	1.03990	-100.47	31.253	1.02010	-82.67
70.231	1.03988		30.915	1.02060	-82.60
66.918	1.03890	-97.82	28.664	1.01820	-81.95
66.440	1.03894	-95.63	26.930	1.01725	-81.78
63.110	1.03846	-94.02	25.869	1.01588	-81.67
62.131	1.03728	-93.69	21.235	1.01300	-80.64
61.895	1.03765	-95.12	20.995	1.01299	-80.06
58.250	1.03614	-91.27	15.156	1.00880	-80.99
56.245	1.03603	-89.27	13.027	1.00492	-79.79
54.934	1.03463	-90.12	11.579	1.00611	-79.43
53.750	1.03428	-86.91	10.061	1.00611	-78.66
51.777	1.03278	-89.03	9.971	1.00494	-79.20
50.513	1.03194	-88.19	7.441	1.00317	-79.94
48.062	1.03065	-86.23	6.622	1.00276	-79.25
47.629	1.03131		5.006	1.00153	-80.48
47.412	1.03037	-86.79	2.504	0.99970	-83.15

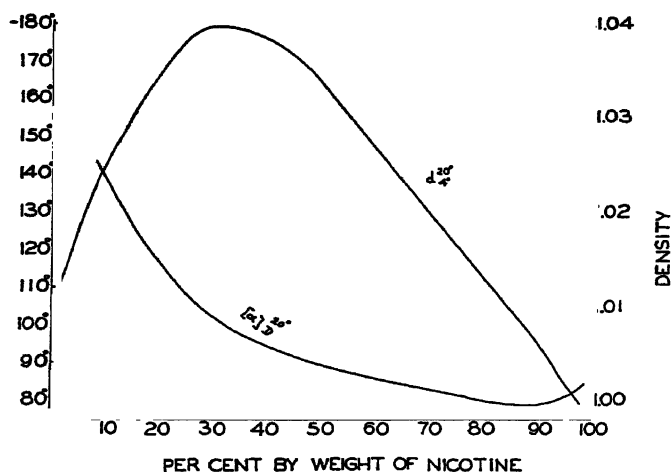


FIG. 7. Density and specific rotation of the system nicotine-water

TABLE 13
Density of the system nicotine-water (19)

NICOTINE	0°C.	10°C.	20°C.	30°C.	40°C.
100.00	1.0258	1.0175	1.0096	1.0014	0.9936
93.64	1.0390	1.0304	1.0218	1.0133	1.0049
83.66	1.0517	1.0430	1.0340	1.0248	1.0150
69.88	1.0577	1.0496	1.0402	1.0310	1.0217
60.07	1.0588	1.0486	1.0402	1.0316	1.0230
51.60	1.0507	1.0442	1.0367	1.0299	1.0210
35.13	1.0342	1.0296	1.0241	1.0181	1.0118
15.82	1.0148	1.0128	1.0098	1.0058	1.0012

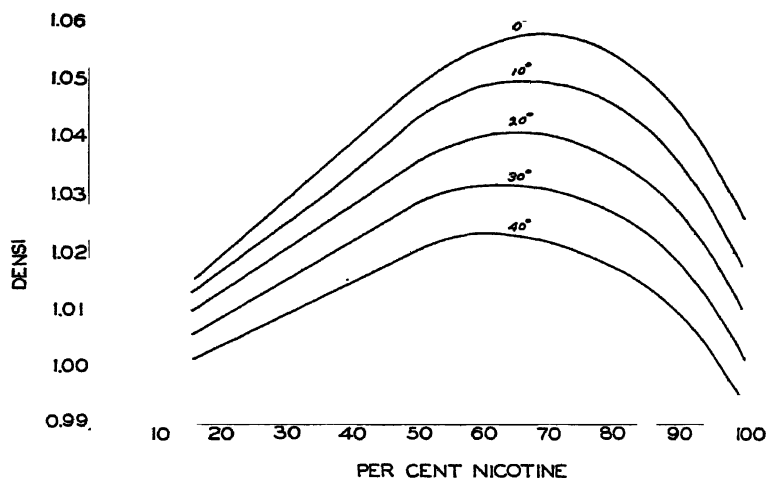


Fig. 8. Density of the system nicotine-water

curves at the lower temperatures (figure 10) show a maximum value which tends to disappear as the lower temperature limit for partial miscibility (about 60°C.) is approached.

Sata (203) determined the densities of the conjugate solutions at various temperatures between 65° and 120°C. At lower temperatures the nicotine phase is the heavier, but reversal occurs at 96°C. and an 8-shaped curve is

TABLE 14
Coefficient of contraction of nicotine-water mixtures (19)

PER CENT NICOTINE	COEFFICIENT OF CONTRACTION				
	0°C.	10°C.	20°C.	30°C.	40°C.
93.63	0.01433	0.01364	0.01266	0.01201	0.01152
83.66	0.02876	0.02730	0.02543	0.02366	0.02225
69.88	0.03770	0.03578	0.03274	0.03032	0.02805
60.07	0.03840	0.03655	0.03382	0.03143	0.02935
51.60	0.03585	0.03394	0.03159	0.02945	0.02736
35.13	0.02458	0.02310	0.02150	0.02001	0.01895
15.82	0.01022	0.01028	0.00972	0.00913	0.00880

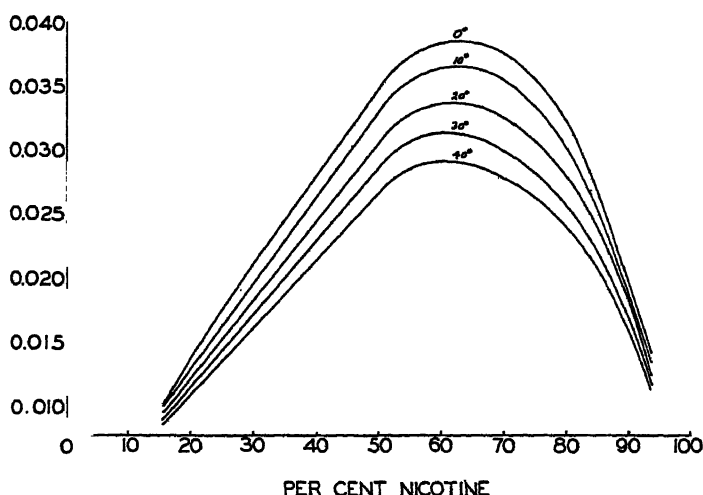


Fig. 9. Coefficient of contraction of the system nicotine-water

obtained on plotting densities of the conjugate against temperature (see table 16 and figure 11).

The curve in figure 12 represents the relation between the percentage composition and the specific rotation of nicotine solutions (see table 17) and may be regarded as built up of three different portions. The points of intersection would lie at about 6 and 69 per cent. The addition of water

TABLE 15
Density of the system nicotine-water (203)

VOLUME PER CENT NICOTINE	DENSITY			
	30°C.	40°C.	50°C.	60°C.
0	0.99567	0.99225	0.98807	0.98324
10	1.00234	0.99856	0.99453	0.98968
20	1.00888	1.00410	0.99886	0.99353
30	1.01513	1.00953	1.00381	0.99755
40	1.02118	1.01449	1.00784	1.00065
50	1.02646	1.01903	1.01033	1.00299
60	1.03023	1.02156	1.01280	1.00407
70	1.03101	1.02228	1.01247	1.00406
80	1.02788	1.01830	1.00875	0.99970
90	1.01773	1.00979	1.00132	0.99280
100	1.00124	0.99383	0.98630	0.97890

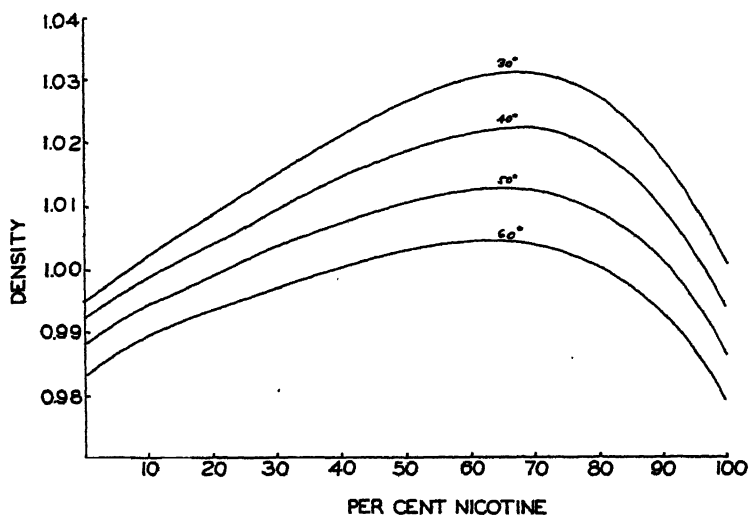


Fig. 10. Density of the system nicotine-water

TABLE 16
Density of the system nicotine-water

TEMPERATURE	UPPER PHASE (WITH NICOTINE- SATURATED WATER LAYER)	LOWER PHASE (WITH WATER- SATURATED NICOTINE LAYER)	TEMPERATURE	LOWER PHASE (WITH NICOTINE- SATURATED WATER LAYER)	UPPER PHASE (WITH WATER- SATURATED NICOTINE LAYER)
°C.			°C.		
65	0.98918	0.99907	95	0.96709	0.96744
70	0.98467	0.99491	100	0.96429	0.96190
75	0.98077	0.98904	105	0.96070	0.95760
80	0.97761	0.98438	110	0.95710	0.95210
85	0.97397	0.97832	115	0.95340	0.94770
90	0.97103	0.97368	120	0.94970	0.94320

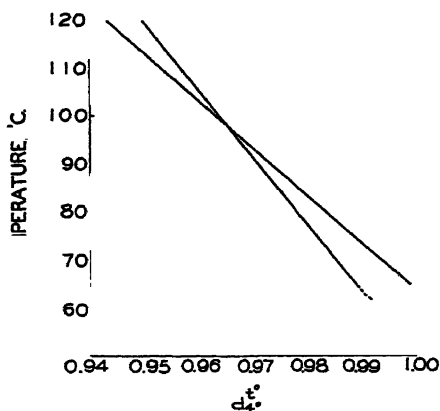


FIG. 11. Density of the system nicotine-water

TABLE 17

Specific gravity and specific rotation of the system nicotine-water (194)

PER CENT NICOTINE	SPECIFIC GRAVITY 20°/4°	$[\alpha]_D^{20^\circ}$	PER CENT NICOTINE	SPECIFIC GRAVITY 20°/4°	$[\alpha]_D^{20^\circ}$
100.000	1.0095	-164.91°	49.301	1.0329	-86.49°
86.889	1.0299	-129.19	35.579	1.0238	-82.17
75.131	1.0394	-103.75	20.746	1.0132	-79.08
72.665	1.0398	-100.27	10.191	1.0054	-77.55
71.315	1.0399	-101.60	9.099	1.0046	-77.56
69.117	1.0402	-99.13	8.167	1.0037	-77.44
67.534	1.0397	-97.79	6.340	1.0026	-77.54
64.921	1.0396	-95.21	4.733	1.0015	-77.34
62.812	1.0391	-93.76	2.545	1.0000	-77.64
59.653	1.0382	-91.64	1.049	0.9990	-78.06
55.401	1.0364	-89.27	0.6175	0.9987	-78.42

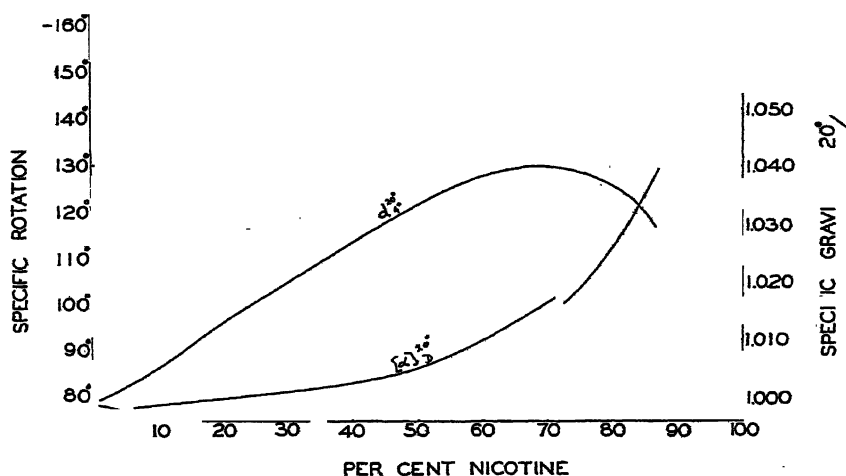


FIG. 12. Specific gravity and specific rotation of the system nicotine-water

to nicotine at first produces a very rapid fall in the rotation, but when the concentration of the nicotine reaches 69 per cent, the fall becomes less rapid, and from 6 per cent onwards there is a rise in the rotation with increasing dilution. The solution containing about 69 per cent of nicotine is the one that possesses the maximum density, and this point also coin-

TABLE 18
Density of the system nicotine-alcohol

PER CENT NICOTINE	$d_4^{20^\circ}$ (118)	GRAMS NICOTINE PER 100 G. METHYL ALCOHOL	$d_4^{20^\circ}$ (268)
90.09	0.9884	81.233	0.97073
74.93	0.9536	65.111	0.93523
59.93	0.9200	47.611	0.89634
45.08	0.8875	25.213	0.84703
30.03	0.8554	12.066	0.81901
14.96	0.8251	11.920	0.81856
		6.031	0.80639
		3.444	0.80106

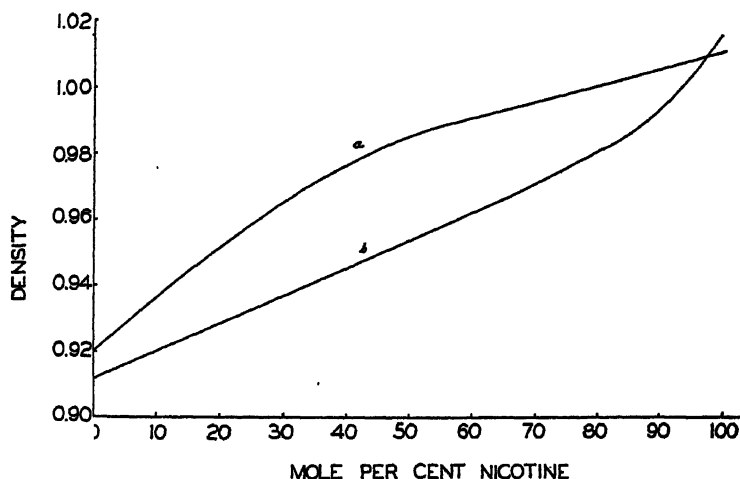


FIG. 13. Density of (a) the system nicotine-2,4-dimethylpyrrole and (b) the system nicotine-2,4-dimethyl-3-ethylpyrrole (cryptopyrrole).

cides with the maximum volume concentration. The solution approximates the composition $C_{10}H_{14}N_2 \cdot 4H_2O$, and Pflüger and Glücksmann (194) are inclined to attribute the peculiarities noticed in nicotine solution to the existence of hydrates.

(b) *Alcohol and nicotine.* Values for the density of mixtures of nicotine and ethyl alcohol are given in table 18.

(c) *Mixtures of 2,4-dimethylpyrrole and 2,4-dimethyl-3-ethylpyrrole with nicotine.* The density curve for a mixture of 2,4-dimethylpyrrole with nicotine (2 moles of 2,4-dimethylpyrrole:1 mole of nicotine) shows a clearly convex sag, whereas that for a mixture of 2,4-dimethyl-3-ethyl-

TABLE 19
Density and specific rotation of the system alcohol-water-nicotine (74)

PERCENTAGE		GRAMS OF NICOTINE PER 100 CC. OF SOLUTION AT 20°C.	DENSITY OF THE SOLUTION AT 0°C.	SPECIFIC ROTATION [α]
Alcohol	Water			
99.330	0.670	12.0167	0.83993	-136.44°
88.848	11.152	12.3770	0.86427	-121.91
78.880	21.120	12.1965	0.88755	-113.40
68.855	31.145	12.1300	0.90780	-108.31
59.207	40.793	12.4175	0.92790	-103.99
50.133	49.867	12.1597	0.94418	-99.09
40.273	59.727	12.1342	0.96309	-94.79
21.233	78.767	12.0189	0.98940	-84.08

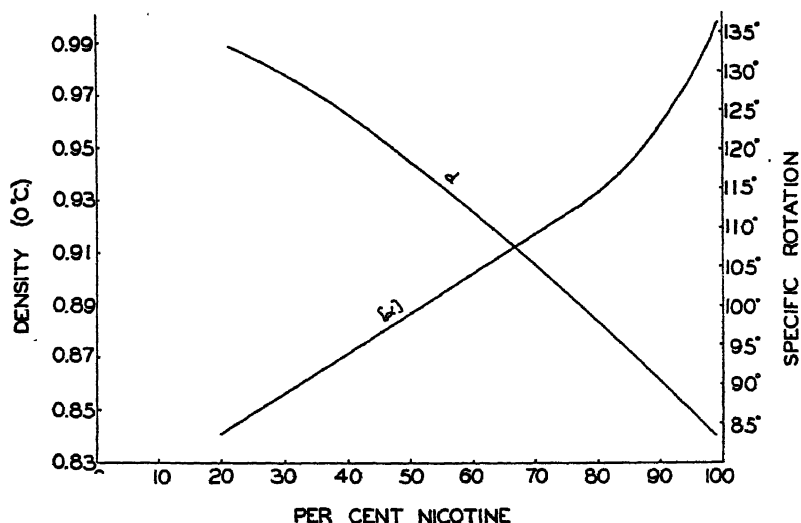


FIG. 14. Density and specific rotation of the system alcohol-water-nicotine

pyrrole (cryptopyrrole) with nicotine (2 moles of cryptopyrrole:1 mole of nicotine) is faintly concave, as shown in figure 13 (54).

(d) *Alcohol, water, and nicotine.* Values for the density and specific rotation of mixtures of ethyl alcohol, water, and pyridine are given in table 19 and represented graphically in figure 14.

13. Thermochemical and dissociation constants

The *heat of combustion* of nicotine at constant volume at 12°C. is 1426.6 Cal.; at constant pressure, 1428.0 Cal. (14, 15); kg.-cal._{15°} (exptl.), 1427.7; kilojoules, 5974.9 (102). Its *heat of formation* from the elements is as follows: (liq) -1.9 Cal.; (dissolved) 5.15 Cal. (14, 15). The *heat of solution* of nicotine is 7.05 Cal. at 11°C. (14, 15) and 6.56 Cal. at 14°C. (44). The *specific heat* of nicotine was found to be 0.420 Cal. (45). Its *molecular heat of solution* is 6.56 Cal. (45); its *heat of dissolution* is 6.6 Cal. at about 15°C. (44). The *heat of neutralization* of nicotine by hydrochloric acid is as follows: first equivalent = 8.05 Cal.; second equivalent = 3.47 Cal. (44). Its *total heat of neutralization* by 4 moles of hydrochloric acid = 12.06 Cal. With sulfuric acid, the first equivalent = 9.54 Cal. and the total heat of neutralization by excess acid = 13.46 Cal. It is evident that the two basic functions have very different energies, a fact which is also shown by color reagents. With litmus as indicator, nicotine has only one basic function, but with dimethyl orange it has two (44). The dissociation constants of nicotine at 15°C. are as follows: $K_1 = 7 \times 10^{-7}$; $K_2 = 1.4 \times 10^{-11}$ (122). Craig and Hixon (48) obtained a value of 9×10^{-7} potentiometrically, and explained the lower value as due to the pyridyl radical being extremely negative. Mendel and Vickery (136) and Vickery and Pucher (249) found that the apparent dissociation constants of nicotine are as follows at 20°C.: $pK_{a1}^1 = 3.22$; $pK_{a2}^1 = 8.11$.

14. Electrometric titration

At a temperature of 26°C., 10 cc. of a 1.333 *N* solution of nicotine was titrated with 1 *N* hydrochloric acid. The neutralization points were at 320 drops, pH = 5.52, and at 640 drops, pH = 1.52 (130). The first section of the potential curve shows the changes produced by the neutralization of the pyrrolidine nitrogen and has the form characteristic of the neutralization of a weak base. The second section shows the neutralization of the pyridyl nitrogen; but this is so weak that the possible formation of a pseudo-base is of no importance, and no drift was observed in the strongly acid solutions in which the dichloride is formed.

The potential curve was plotted with the help of a hydrogen electrode, while drops of 1 *N* hydrochloric acid from a buret were being added to the base in a glass vessel closed with a rubber bung and provided with a glass stirrer. The glass vessel was connected to a beaker of saturated potassium chloride by an agar-agar bridge, also saturated with hydrochloric acid. The cell was completed by a saturated calomel electrode dipping into the beaker containing potassium chloride, and its E.M.F. was determined at room temperature by means of a potentiometer calibrated to read directly in millivolts. The results are shown in figure 15.

The basic dissociation constants of the two nitrogen atoms were then deduced from the values of the pH at half-neutralization as follows: pyridyl nitrogen, 7.94×10^{-12} ; pyrrolidine nitrogen, 4.90×10^{-7} .

Dubrisay (57) determined the exact neutralization of nicotine to occur at a pH of 5.45, suitable indicators being bromocresol purple, sulfoalizarin, *p*-nitrophenol, and methyl red. Nicotine with methyl red gave a pH of 5.26 as the neutralization point in electrometric titration (255). Schick

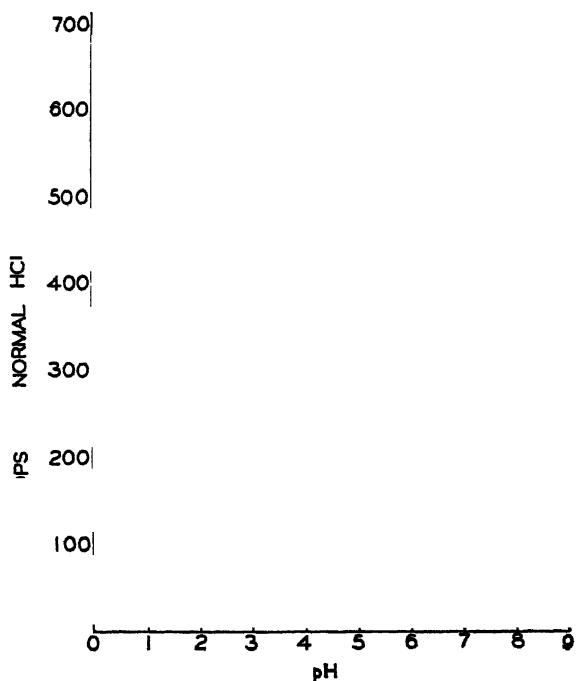


FIG. 15. Electrometric titration of nicotine

and Hatos (204) found that methyl red gave a much sharper and more accurate end point than iodeosin in the titration of nicotine.

15. Specific rotation

The following values have been reported for the specific rotation of nicotine: -160.93° (178); -161.29° (146); -161.50° (276); -161.55° (118, 171, 187, 192, 267); -162.84° (69); -163.0° (52); -163.19° (197); -164.0° (89); -164.18° (85); -164.91° (194); -166.39° (178); -166.77° (197); -167.78° , -168.02° (168); -168.20° (131); -169.40° , -168.52° , -168.61° , -168.66° (98); -168.84° , -169.06° , -169.17° (129); -169.22°

(197); -169.23° , -169.30° , -169.35° (129); -169.40° (235); -169.50° (131); -169.54° (197) (see also table 20).

The data in table 21 (119) show that the specific rotation of nicotine at first suffers a very sharp decrease with increase in the amount of water added, but that the decrease later becomes gradually less. The strongly bowed curve shown in figure 16 is a limb of an hyperbola.

The data of table 22 show that, at temperatures of 5° and 15°C. , a minimum specific rotation (marked %) occurs when the amount of nicotine

TABLE 20
Specific rotation of nicotine (268)

TEMPERATURE	$[\alpha]_D^t$	LIGHT	$[\alpha]^{14.5^\circ}$
$^\circ\text{C.}$			
17.1	-163.71°	Red*	-125.4°
20.0	-163.89	Yellow	-163.4
42.7	-165.25	Green	-204.5
61.6	-166.05	Light blue	-294.7
82.1	-166.52	Dark blue	-336.8
99.0	-166.56		
121.3	-166.61		

* The colors refer to Landolt's ray filter.

TABLE 21
Specific rotation of the system nicotine-water

PER CENT NICOTINE	$[\alpha]$
89.9155	-133.85°
78.3920	-109.53
65.8872	-94.24
53.4750	-86.58
34.2854	-80.78
17.6793	-76.94
16.3356	-76.88
8.9731	-75.53

is about 10 per cent, and that at a temperature of 20°C. this minimum is found in the solution of about 8 per cent strength. From the molecular weight determination it is seen that nicotine in very dilute solution is present as a normal molecule, while in more concentrated solutions hydrates, and probably molecular aggregations, exist. A much lower rotating power must be ascribed to these than to pure nicotine, but if they are broken up by increasing dilution, as suggested by the decrease in the molecular weight, more and more nicotine appears; thus the decrease in

the specific rotation is gradually arrested and finally an increase in the rotation must follow.

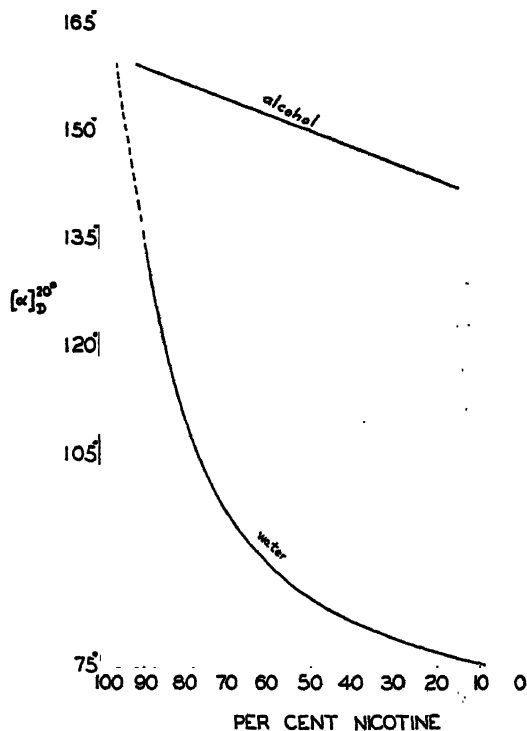


Fig. 16. Specific rotation of nicotine in (a) alcohol and (b) water

TABLE 22

Specific rotation of the system nicotine-water (85)

PER CENT NICOTINE	$[\alpha]_D^{15^\circ}$	$[\alpha]_D^{18^\circ}$	$[\alpha]_D^{20^\circ}$
15.592	-73.39°	-76.18°	-77.59°
11.206	-73.05	-75.96	-77.01
10.258	-72.78 (#)	-75.59 (#)	-76.89
8.307	-73.07	-75.76	-76.84 (#)
5.700	-73.81	-76.00	-76.96
3.016	-74.46	-76.27	-77.25
2.042	-74.74	-76.35	-77.32
1.061	-74.79	-76.83	-77.66

Přibram (192) observed that an aqueous solution containing 20.169 per cent of nicotine and having a density of 1.0149 exhibited, when it was

allowed to remain in the polarization tube, the following increase in rotation:

	$[\alpha]_D^{20^\circ}$
Fresh solution.....	-87.81°
After 12 hr.....	-93.13
After 18 hr.....	-96.55
After 48 hr.....	-96.56

TABLE 23
Specific rotation of nicotine in organic solvents (267)

SOLVENT	PER CENT NICOTINE	$[\alpha]_D$	SOLVENT	PER CENT NICOTINE	$[\alpha]_D$
Acetone.....	93.7	-163.68°	Propyl alcohol	88.55	-160.76°
	56.5	-164.65		60.01	-156.34
	31.6	-163.56		25.37	-149.92
	20.5	-163.46		14.07	-147.35
	14.7	-163.25		9.65	-146.35
	3.1	-162.73		7.46	-145.89
				1.83	-143.67
Ether.....	82.3	-164.17°	Aniline.....	84.79	-160.78°
	62.9	-163.50		68.42	-157.65
	37.1	-162.78		50.24	-156.86
	18.2	-162.36		27.08	-156.55
	13.3	-162.26		15.97	-156.45
	5.7	-161.72		6.00	-156.41
Benzene.....	84.4	-164.29°	Toluidine....	84.96	-161.40°
	48.0	-164.14		64.17	-158.55
	25.5	-164.10		34.36	-157.65
	19.0	-163.95		19.98	-157.32
	16.4	-163.88		12.34	-156.88
	8.5	-163.67		4.60	-156.55
Ethyl alcohol..	90.09	-158.65°			
	74.93	-154.92			
	59.93	-151.78			
	45.08	-148.81			
	30.03	-145.42			
	14.96	-141.60			

According to Přibram, this phenomenon depends on the formation of a hydrate, since nicotine and water mix with marked evolution of heat. In a freshly prepared 20 per cent solution the conversion does not seem to be

completed at once, but requires a certain time. From the increase in the rotation it must be concluded that the hydrate is more active than the pure base.

TABLE 24
Specific rotation of nicotine

λ	T	OBSERVED ROTATION	$[R]_T^\lambda$
	$^{\circ}\text{C.}$		
480	22.3°	-197.83°	-251.81°
	-81.5	-191.04	-223.67
508.6	22.4	-169.76	-216.08
	-81.5	-163.38	-191.27
546.1	22.4	-141.96	-180.70
	-72.5	-138.25	-162.85
	-81.5	-136.02	-159.24
	-89.7	-134.69	-156.61
	-45.0	-142.24	-171.54
	-30.0	-142.86	-174.42
	-15.0	-143.15	-176.90
	-5.0	-143.10	-178.20
	5.0	-142.92	-179.40
589.3	22.7	-117.80	-149.97
	-71.5	-114.44	-135.02
	-82.1	-112.57	-131.73
	-89.0	-111.19	-129.40
643.8	22.5	-95.36	-121.38
	-71.2	-92.82	-109.51
	-80.7	-91.34	-106.98
670.8	22.5	-86.76	-110.43
	-72.2	-84.18	-99.23
	-81.6	-82.84	-96.98

As shown in table 23, the specific rotation of nicotine decreases as the dilution with ethyl alcohol increases. The graphic construction in figure 16 leads to a straight line.

Molby (142) measured the specific rotation of nicotine for six different wave lengths and through a low temperature range. In table 24, the column headed $[R]_T^\lambda$ gives the specific rotation for the corresponding temperature as obtained by the equation

$$[R]_T^\lambda = \text{observed rotation}/LD$$

in which L is the length of the liquid column expressed in decimeters and D is the density at the corresponding temperature.

In an equimolecular mixture of nicotine and glacial acetic acid, treated with water, Gennari (69) observed the appearance of anomalous dispersion, but only within very narrow limits of concentration. The specific rotation of nicotine in mixtures of acetic acid and water at 20°C. was found by means of Landolt's ray-filter method (see table 25); for yellow light, however, the sodium flame was used.

Landolt (119) points out that pure nicotine and the mixture of this with one mole of acetic acid possess a normal levorotation, which increases

TABLE 25
Specific rotation of the system nicotine-acetic acid-water at 20°C.

MIXTURES	$[\alpha]_{\text{red}}$	$[\alpha]_{\text{D}}$	$[\alpha]_{\text{green}}$	$[\alpha]_{\text{light blue}}$	$[\alpha]_{\text{dark blue}}$
Equimolecular mixtures of glacial acetic acid and nicotine.....	-55.63°	-74.18°	-95.22°	-120.3°	-143.54°
Mixtures of the same with water:					
original mixture, water					
per cent per cent					
82.806 17.194	-11.79°	-16.00°	-20.27°		
81.259 18.741	-9.20	-12.57	-16.83	-21.99°	-26.68°
78.866 21.134	-4.13	-5.82	-8.20	-10.92	
77.844 22.156	-1.70	-2.72	-4.39	-6.63	-9.93
77.450 22.550	-0.12	-0.54	-1.48	-2.80	-5.00
76.390 23.610	+0.78	+0.52		-0.75	
76.300 23.700	1.36	0.98	+0.74		-1.40
76.230 23.770	5.90	7.44	8.85	+9.87	
76.100 23.900	12.92	16.74	20.72	24.56	+28.00

with the refrangibility of the light. As nicotine acetate rotates to the right, it appears that a part of the nicotine in the mixture exists in the uncombined condition.

The peculiarities in dispersion which appear when water is added to the equimolecular mixture of acetic acid and nicotine can best be seen from the diagram in figure 17. This extends from the red to the light blue, as $[\alpha]$ for dark blue had to be omitted on account of incompleteness in the observations. It will be seen: (1) That in mixtures with 18.74 and 22.55 per cent of water there is normal levorotation for the different colors, and that this decreases (the dispersion also) with increase in the amount of water. (2) That the solutions containing from 23.77 to 23.90 per cent of

water possess dextrorotatory power, which, with the dispersion, is increased in a marked degree by a slight increase in the amount of water. (9) That with an amount of water varying from 23.61 to 23.70 per cent, the rotation and also the dispersion are very small, the first solution showing complete inactivity for green rays and the second for light blue rays. Here then is a case of the appearance of a minimum of rotation with increasing refrangibility of the light.

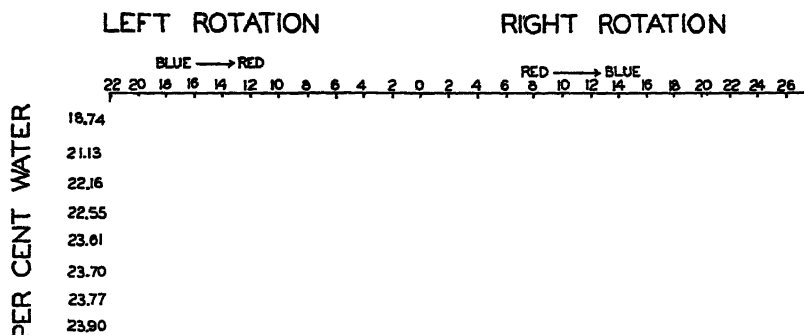


FIG. 17. Dispersion of nicotine-acetic acid (equimolecular solution) in water

A sample of nicotine which possessed the specific rotation $[\alpha]_D^{20} = -164^\circ$ gave, when dissolved in benzene, the following values (85):

PER CENT NICOTINE	$[\alpha]_D^{20}$	PER CENT NICOTINE	$[\alpha]_D^{20}$
84.36	-164.29°	19.00	-163.95°
48.02	-164.14	16.36	-163.88
25.47	-164.10	8.52	-163.67

There is here but a very small change in the specific rotation, amounting to only 0.2 per cent with change in concentration.

In like manner (see table 23) the specific rotation of nicotine remains almost unchanged when it is dissolved in ether or acetone, while with aniline or toluidine as solvent there is a slight decrease, to about $[\alpha]_D^{20} = -156.5^\circ$. The decrease in ethyl alcohol or propyl alcohol is somewhat more, while in water it is very marked. Tables 26, 27, and 28 present data for the rotatory dispersion of nicotine in various solvents. The data of table 28 are shown in graphic form in figure 18.

Milward-Liquier and Descamps (141) conclude that aqueous nicotine solutions to which hydrochloric acid has been added until one of the basic functions has been completely neutralized behave like mixtures of

two optically active constituents, the dispersions of which are different and the proportions of which vary with the pH of the solution. One of the

TABLE 26
Specific rotation of nicotine in various solvents

SOLVENT	PER CENT NICOTINE	$[\alpha]_D^{20}$
Water.....	14.095	-70.0°
Methyl alcohol.....	11.920	-118.0
Ethyl alcohol.....	10.518	-131.0
Isobutyl alcohol.....	10.732	-141.0
Benzene.....	9.647	-160.0
Chloroform.....	7.824	-157.0
Ethylene bromide.....	4.676	-174.0
Aniline.....	7.995	-156.0

TABLE 27
Rotatory dispersion of nicotine at 20°C. (129)
Specific rotation $[\alpha]_{\text{MCL}} = -204.14^\circ$

VISIBLE SERIES		PHOTOGRAPHIC SERIES	
λ in Å.	α ($l = 6$)	λ in Å.	α ($l = 1$)
Li 6707.8	-756.89°	Fe 4272	-399.5°
Cd 6438.5	-832.25	Fe 4251	-405.8
Zn 6362.3	-855.35	Fe 4202	-419.8
Li 6103.7	-943.75	Fe 4199	-2531.9*
Na 5892.9	-1025.71	Fe 4176	-428.0
Cu 5782.1	-1074.81	Fe 4145	-438.5
Hg 5780.1	-1076.14	Fe 4132	-442.9
Cu 5700.2	-1112.92	Fe 4072	-462.8
Hg 5460.7	-1237.38	Fe 4062	-2804.4*
Cu 5218.2	-1387.58	Fe 4046	-472.7
Ag 5209.0	-1394.17	Fe 4005	-488.7
Cu 5153.3	-1432.91	Fe 3967	-504.0
Cu 5105.5	-1467.84	Fe 3953	-510.5
Cd 5085.8	-1482.41	Fe 3903	-531.3
Zn 4810.5	-1717.49	Fe 3887	-539.4
Cd 4799.9	-1727.78		
Zn 4722.2	-1806.25		
Hg 4358.3	-2266.31		

* $l = 6$.

constituents is the non-electrolytically dissociated molecule and the other is the optically active ion. These conclusions hold both in the visible and in the ultraviolet range.

Tate and Warren (235) find that existing data concerning the optical rotation of nicotine in aqueous solution are conflicting. The earlier work of Popovici (187) and of Příbram (192) indicates irregularities in the specific rotation at low concentrations. Jephcott (98), in the course of an examination of solutions ranging in concentration from 2.5 to 100 per cent, found a minimum value of $[\alpha]_D^{20} = -78.7^\circ$ at 10 per cent, the value rising

TABLE 28

Rotatory dispersion of nicotine at 20°C. in (a) acetone, (b) water, (c) acetic acid, and (d) butyric acid (129)

(a) 28.7784 g. OF NICOTINE PER 100 g. OF SOLUTION		(b) 25.4983 g. OF NICOTINE PER 100 g. OF SOLUTION		(c) 15.5230 g. OF NICOTINE PER 100 g. OF SOLUTION		(d) 14.0798 g. OF NICOTINE PER 100 g. OF SOLUTION	
λ in Å.	α ($l = 4$)	λ in Å.	α ($l = 6$)	λ in Å.	α ($l = 6$)	λ in Å.	α ($l = 4$)
Li 6708	-126.03°	Li 6708	-91.83°	Li 6708	+28.97°	Li 6708	+16.54°
Zn 6363	141.81	Cd 6438	101.17	Cd 6438	31.76	Zn 6363	18.61
Li 6104	156.81	Zn 6363	104.23	Zn 6363	32.54	Li 6104	20.37
Na 5893	169.81	Li 6104	115.04	Li 6104	35.63	Na 5893	21.90
Cu 5782	177.87	Na 5893	125.37	Na 5893	38.60	Hg 5780	22.83
Hg 5782	178.06	Cu 5782	131.35	Hg 5780	40.31	Cu 5700	23.67
Cu 5700	184.17	Hg 5780	131.53	Cu 5700	41.59	Hg 5461	25.89
Hg 5461	204.48	Cu 5700	136.20	Hg 5461	45.89	Cu 5218	28.64
Cu 5218	228.89	Hg 5461	151.95	Cu 5218	50.90	Cu 5153	29.53
Cu 5153	236.61	Cu 5218	171.03	Cu 5153	52.22	Cu 5105	30.20
Cu 5103	242.28	Cu 5153	176.86	Cu 5105	53.35	Zn 4810	34.61
Zn 4811	283.11	Cu 5106	181.36	Cd 5086	53.78	Li 4603 Hg 4358	α ($l = 1$) 9.44 10.66
Zn 2722	297.16	Cd 5086	183.21	Zn 4811	60.77		
Hg 4358	371.56	Zn 4810	213.65	Zn 4722	63.53		
		Cd 4800	214.83	Zn 4680	64.80		
		Zn 4722	225.02				
		Hg 4358	285.03		α ($l = 1$)		
				Li 4602	11.21		
				Hg 4358	12.79		
				Fe 4281	13.28		
				Fe 4202	13.88		
				Fe 4187	14.00		
				Fe 4144	14.33		
				Fe 4132	14.41		

to $[\alpha]_D^{20} = -83.15^\circ$ at 2.5 per cent, which was the most dilute solution examined. Tingle and Ferguson (241) and Lowry and Lloyd (129) found that the optical rotation was directly proportional to the concentration.

Working with a polarimeter using "j" light, Tingle and Ferguson (241) carried out experiments which showed (2) that, for solutions containing between 4 and 0.37 per cent of nicotine, the rotation of aqueous nicotine

solutions is in simple inverse proportion to the concentration; (ii) that the presence of potassium hydroxide and potassium salts has no influence on the rotation of nicotine salts; (iii) that nicotine is not lost by vaporization when an acid solution of nicotine sulfate is evaporated.

In the determination of the rotation values of dilute aqueous nicotine solutions, nicotine which had been freshly distilled in a vacuum was brought to a standard dilution with pure distilled water free from ammonia and carbon dioxide; these solutions were subsequently diluted to lower concentrations. Three such standard solutions were prepared, and

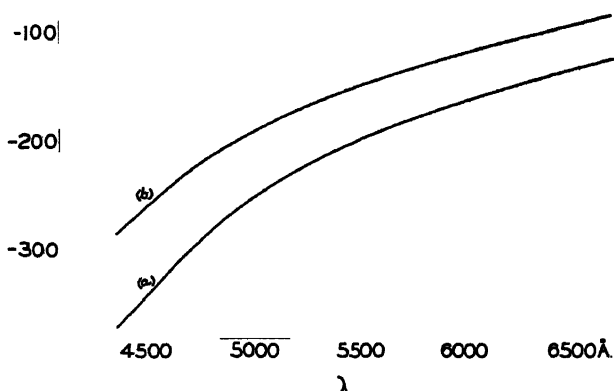


FIG. 18. Rotatory dispersions of nicotine at 20°C. Curve a, 28.7734 g. of nicotine per 100 g. of its solution in acetone; curve b, 25.4983 g. of nicotine per 100 g. of its solution in water; curve c, 15.5230 g. of nicotine per 100 g. of its solution in acetic acid; curve d, 14.0793 g. of nicotine per 100 g. of its solution in butyric acid.

measurements were made with a Hilger polarimeter and a Schmidt and Haensch saccharimeter. The readings were directly proportional to the concentration, thus confirming Tingle and Ferguson's data. The specific rotation was shown to be constant between 0 and 10 per cent, the values determined being $[\alpha]_D^{20} = -79.4^\circ$ and $[\alpha]_{5461}^{20} = -96.1^\circ$.

Figure 19 shows the relation between specific rotation and temperature through the range from -90° to 24°C . with wave length $\lambda = 546.1$. It seems that the specific rotation decreases with decreasing temperature very much more rapidly at the low temperatures than at ordinary temperatures. The liquid was very viscous at -90°C ., this being the lowest tem-

perature at which observations could be made with anything like the length of liquid column used.

A series of observations extending over a period of 2.5 hr. was made with wave length 546.1μ , while the temperature was gradually lowered from 20° to -45.3°C . The results were plotted in the curve as shown in figure

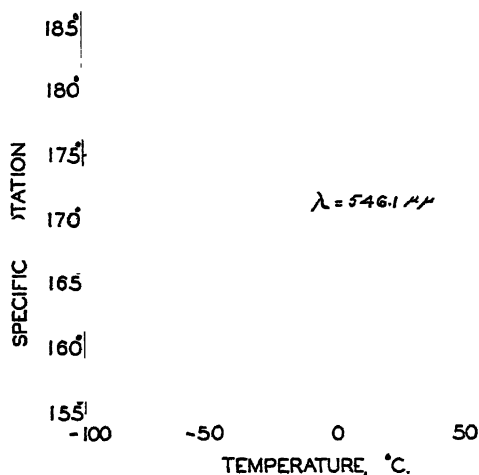


FIG. 19. Relation between specific rotation of nicotine and the temperature

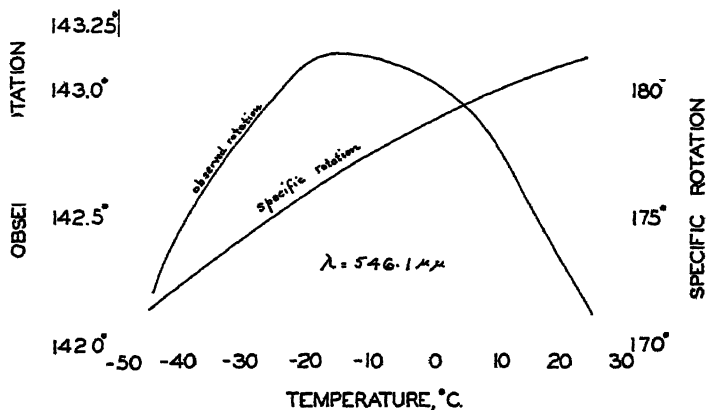


FIG. 20. Observed and specific rotations of nicotine

20 to such a scale as to show the temperature at which an apparent rotation is reached.

Each point plotted is the mean value for five readings of both rotation and thermal E.M.F. The specific rotation at the same temperatures was computed and the curve plotted to show the contrast between observed

rotation and specific rotation. The maximum observed rotation is at a temperature of approximately -15°C .

It is of interest to see graphically a comparison of the relative effects of temperature upon the rotation of different wave lengths. In figure 21 (upper curve) the rotations observed at 22.4°C . are plotted as abscissae and the decrease in the observed rotation at -80°C . as ordinates. In the same figure (lower curve), the specific rotations at room temperature are plotted as abscissae with the decrease in specific rotation, for the corresponding specific rotations at -80°C ., as ordinates. From the measurements with wave lengths at the central part of the spectrum it might therefore appear that the temperature effect is relatively the same for all wave

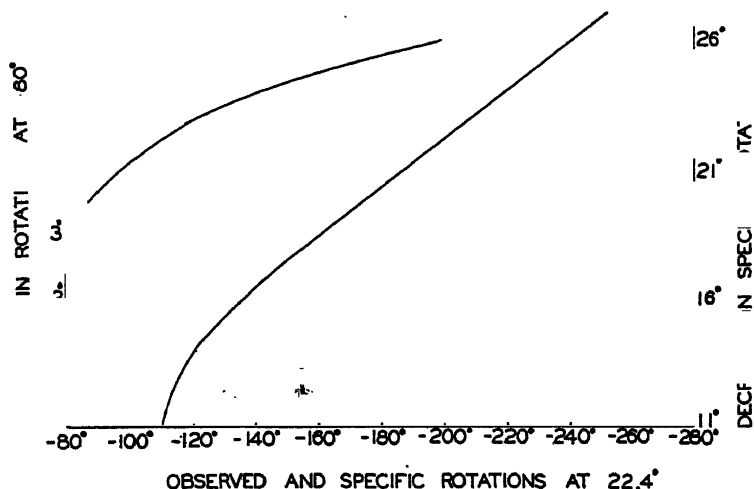


FIG. 21. Observed and specific rotations of nicotine at 22.4°C .

lengths, but the measurements with the longer wave lengths show greater relative decreases (142).

16. Study of anomalous rotatory dispersion (270)

Nicotine is levorotatory and its salts are dextrorotatory. Lowry and Singh (131) have shown that the alkaloid in the homogeneous condition at 20°C . exhibits simple rotatory dispersion in the negative region. Genari (69) observed that an equimolecular mixture of nicotine and glacial acetic acid, within certain limits of dilution with water, exhibits anomaly in the positive region. Nicotine salts are therefore assumed to be of relative configuration *d*.

The rotation of the base is due to the asymmetry of the α -pyrrolidine

carbon atom; in the salts, however, a new center, the asymmetric pyrrolidine nitrogen atom, is involved (125). No decision as to the configuration of the free base is at present possible, for the respective configurations and dominance of the two centers are unknown.

Patterson and Fulton (168) have shown that the rational zero (for nicotine and its sulfate) is 3° , and if this rotation could be realized for the green rays, the rotatory dispersion would be visibly anomalous in the positive region. But a deduction as to the relative configuration of the free base from the data given would require careful consideration of the occurrence and movement of maxima in the negative region in the temperature-rotation curves for the homogeneous base at high temperature, and similarly for the base in inert solvents, and also the construction of separate characteristic diagrams, as well as a composite one.

The fact that nicotine and its sulfate in various solvents fit on the same characteristic diagram suggests that the α -pyrrolidine carbon atom is of relative configuration *d*.

The variation of the diffusion coefficients for the extreme limits of concentration employed at 20°C . for nicotine was 0.456 to 0.273 for 0.1 *N* to 1 *N*. The mean value of the temperature coefficient of diffusion is about 0.016 (153).

17. Magnetic rotatory dispersion (127)

The magnetic rotatory dispersion of nicotine was measured with the same magnet and by the same methods as those of a long series of aliphatic compounds which had been examined by Lowry (124). Readings were made for twelve wave lengths in the visible spectrum.

The results as given in table 29 show: (i) the double deflection observed with a column 1 dm. long, after correction for the effect of the end plates; (ii) the values of Verdet's constant deduced from these readings; and (iii) the ratio of the rotation at any wave length to that at the wave length of the green (Hg) line. The observed ratio $\delta_{4353}/\delta_{5461}$ is 1.664.

18. Refractive dispersion (127)

Measurements of the refractive index of nicotine were made by Gifford and Lowry (72) by the hollow-prism method, but the scope of the work was limited by the strong absorption of light by the thick column of liquid in the prism. The observations were therefore confined to a range of wave lengths in the visible spectrum between 7685 and 4341 Å., although the absorption of the liquid does not become important before 3000 Å. in the ultraviolet. Since the refractive dispersion is most interesting in the immediate neighborhood of the absorption band, it was thought advisable to make a further series of measurements in the ultraviolet, by

the interferometric method which Lowry and Allsopp (126) have developed for measuring the refractive indices of thin films of liquid. Under these more favorable conditions the measurements have been extended to 2864 Å., i.e., within 200 Å. from the head of the absorption band, with a maximum molecular extinction coefficient $\log \epsilon = 3.43$ at 2650 Å. in cyclohexane. Measurements were also made, with an improved Pulfrich refractometer, of the refractive index for twenty-eight wave lengths in

TABLE 29

Magnetic rotatory power of nicotine at 20°C.

Double rotation, 2α , of water at 5461 Å. = 7.10° ; Verdet's constant for water at 5461 Å. = 0.01534 min./cm. gauss

λ in Å.	2α (CORR.)	VERDET'S CONSTANT δ	$\frac{\delta}{\delta_{5461}}$	λ in Å.	2α (CORR.)	VERDET'S CONSTANT δ	$\frac{\delta}{\delta_{5461}}$
		min./cm. gauss				min./cm. gauss	
Li 6707.86	7.60°	0.01643	0.593	Ag 5467.71	12.79°	0.02764	0.998
Cd 6438.47	8.47	0.01831	0.661	Hg 5460.73	12.82	0.02770	1.000
Zn 6326.35	8.85	0.01911	0.690	Ag 5209.04	14.21	0.03072	1.109
Li 6103.60	9.72	0.02100	0.758	Cd 4799.91	17.09	0.03689	1.332
Na 5892.62	10.53	0.02274	0.821	Li 4602.50	18.67	0.04033	1.456
Hg 5780.13	11.00	0.02379	0.859	Hg 4358.34	21.34	0.04609	1.664

TABLE 30

Refractive index of nicotine at 15°C. (72)

$[\alpha]_{5461}^{20} = -205^\circ$ in a 1-dm. tube

WAVE LENGTH	n	WAVE LENGTH	n
A' 7685 K $_{\alpha}$	1.52061	E 5270 Fe	1.53514
B' 7065 He	1.52294	F 4851 H $_{\beta}$	1.54035
6708 Li	1.52452	Φ 4678 Cd	1.54317
C 6563 H $_{\alpha}$	1.52526	4471 He	1.54699
D 5898 Na	1.52939	4413 Cd	1.54808
A 5608 Pb	1.53177	G 4341 H $_{\gamma}$	1.54935
5461 Hg	1.53291		

the visible spectrum. These are less exact than Gifford's measurements, since they are only valid to the fourth decimal place, but they are probably more trustworthy, by reason of a closer control of temperature and more recent purification of the material. These data are plotted in the curve shown in figure 22.

Tate and Warren (235), using the Zeiss immersion refractometer at 15.5°C., obtained the values shown in table 31 for nicotine-water solutions.

19. Absorption spectra (195)

The optical properties of nicotine appear to be controlled by two characteristic frequencies: the first corresponds to the familiar absorption band in the middle ultraviolet, with a maximum $\log \epsilon = 3.43$ at 2640 \AA . in cyclohexane; the second characteristic frequency is in the Schumann

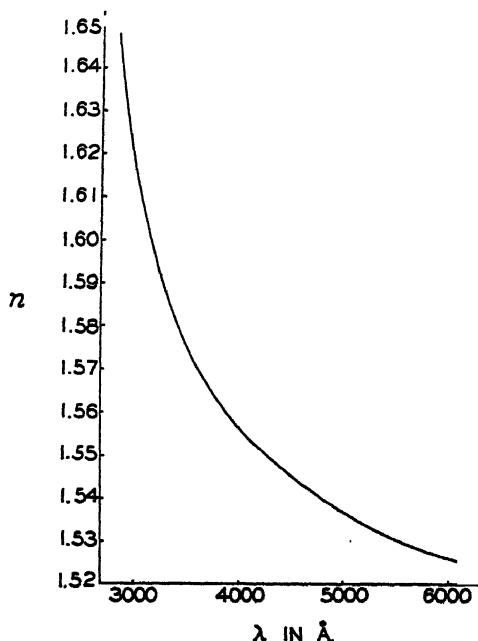
FIG. 22. Refractive indices of nicotine at 20°C .

TABLE 31

Refractive index of the system nicotine-water

CONCENTRATION, GRAMS OF NICOTINE PER 100 ML. OF SOLUTION	n	CONCENTRATION, GRAMS OF NICOTINE PER 100 ML. OF SOLUTION	n
2.652	1.33873	7.293	1.34817
3.646	1.34086	7.707	1.34906
4.580	1.34283	10.028	1.35384
5.574	1.34489	10.160	1.35403

region at 1326 to 1740 \AA . The position and magnitude of this absorption band have not been observed directly, since it lies beyond the limit of transmission of light by air or by quartz, but it appears to be far more intense than the bands which have been recorded in the more accessible part of the ultraviolet. Direct evidence of the existence of a second absorption band is provided by the development of a strong general absorp-

tion in all those derivatives of nicotine in which the pyridyl nitrogen is ionized, although it is not observed either in nicotine itself or in those derivatives in which only the pyrrolidyl nitrogen is ionized. This general absorption may represent the foot of the intense band in the Schumann region, but it might be due to a third absorption band in an intermediate position near the edge of the Schumann region.

The relative influence of these two absorption bands varies greatly in different optical properties and in different derivatives. The most remarkable fact is the dominance by the near ultraviolet band of the optical rotatory power of nicotine, which can be expressed very nearly by a single term of Drude's equation, with $\lambda_0 = 2450 \text{ \AA.}$ (129). This predominance persists in chloronicotine and in methylnicotine (128), where both nitrogen atoms are tetravalent and neutral, as well as in nicotine isomethiodide (129) where the pyrrolidine nitrogen remains neutral, although the pyridyl

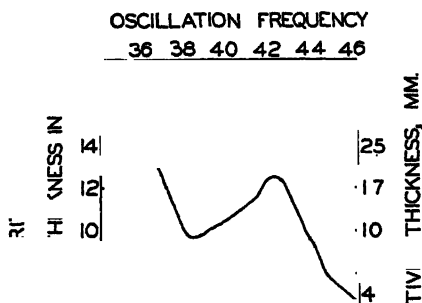


FIG. 23, Absorption of nicotine vapor: $N/1000$ solution of nicotine

nitrogen is ionized by the addition of a methyl radical. When, however, the pyrrolidyl nitrogen, which is attached directly to the asymmetric carbon atom, is ionized, the influence of the ultraviolet absorption band disappears abruptly.

Hartley (82) examined the absorption of a 1-mm. layer of a 10 per cent nicotine-alcohol solution and found that there was general absorption at about $\lambda 2800$; through a thickness of 5 mm. the rays were absorbed at about $\lambda 3132$, but no band was observed. In the author's experiments with $N/10$ solutions through a layer 2 mm. thick, the rays were absorbed at $\lambda 2800$, and through a layer 30 mm. thick at $\lambda 3050$; for $N/100$ solutions, through a 2-mm. layer, the rays were absorbed at $\lambda 2720$ and, through a 30-mm. layer, at $\lambda 2810$, and no band was observed. Upon the examination of $N/1000$ solutions, a band was discovered; its absorption curve has been represented in figure 23. The head of the band of nicotine, expressed in oscillation frequencies, is 3820.

20. *Selective solvent action of nicotine-water solutions on a third substance*

The selective solvent action of the system nicotine-water is shown in table 32.

TABLE 32

Selective solvent action of the system nicotine-water (271)

40 cc. of nicotine in 100 cc. of water; density, 1.007; freezing point of solution, $-2^{\circ}\text{C}.$;
20 cc. of solution used as solvent

THIRD SUBSTANCE USED	10	δ°
	WEIGHT OF THIRD SUBSTANCE USED	ELEVATION OF THE FREEZING POINT
	<i>grams</i>	$^{\circ}\text{C}.$
Benzene	0.0770	0.0300
	0.1385	0.0430
	0.2130*	0.0720
	0.2977	0.0810
	0.3185	0.0830
Carbon tetrachloride.....	0.0965	0.0100
	0.2020	0.0220
	0.2985*	0.0270
	0.3995	0.0300
	0.5250	0.0310
Chloroform	0.0795	0.0230
	0.1640	0.0360
	0.3020	0.0750
	0.4070*	0.0950
	0.5220	0.1150
	0.6536	0.1260
Methyl salicylate.....	0.0655	0.0430
	0.1430	0.0630
	0.2145	0.1080
	0.2995*	0.1250
	0.3920	0.1250
	0.5070	0.1250
Bromonaphthalene.....	0.0460	0.0450
	0.0975*	0.0600
	0.1375	0.0680
	0.1780	0.0700
	0.2170	0.0700

* The system separates into two liquid phases.

The depression of the freezing point of a solvent produced by the addition of a solute may be diminished by the subsequent addition of a third substance; the diminution may be due to various causes based on the decrease

in the concentration of the solute molecules or to complexes in the solution. When a solute is added to a binary mixed solvent in only one of whose components it is soluble, the freezing point of the constituent in which it is insoluble is always raised. When the concentration of the semi-solute is plotted against the elevation of the freezing point, a continuous curve results with a break at the point of formation of two liquid phases. It thus seems evident that a semi-solute when dissolved in a mixed solvent forms solvents with the constituent in which it is soluble, and as a result raises the freezing point and the vapor pressure of the other constituent.

21. Inhibition phenomena

Rane and Prasad (196) concluded that the effect of nicotine in inhibiting the evolution of hydrogen by the action of hydrochloric acid (see table 33) is somewhat analogous to the phenomenon of passivity.

TABLE 33

Effect of 0.02 g. of nicotine on the rate of solution of iron in hydrochloric acid (196)

TIME	VOLUME OF HYDROGEN EVOLVED	
	Without the alkaloid	On the addition of nicotine
hours	cc.	cc.
1	91.7	
2	143.6	
3	177.4	91.2
4	224.3	109.8
5	286.7	110.4
6	248.4	133.0

G. CHEMICAL PROPERTIES

That nicotine is a feeble base is shown by the fact that dilute aqueous solutions containing the alkaloid and hydrochloric or acetic acid in molecular proportions exhibit a contraction on mixing instead of the augmentation of volume noticed with strong bases and acids. Even when pure nicotine and pure liquid acetic acid are mixed in molecular proportions, a contraction of about 2.5 per cent in volume occurs (146).

Nicotine is a diacid base, since it forms well-crystallized salts with one and two equivalents of acids. It combines with two molecules of an alkyl halide, for example, with two of methyl iodide, forming a methiodide from which moist silver oxide separates nicotine methyl hydroxide; by this behavior nicotine shows itself to be a ditertiary base (7).

1. Oxidation

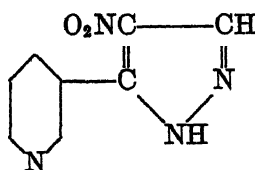
(a) *Autooxidation.* Ciamician and Silber (41) reported the autooxidation of nicotine under the influence of light. Their experiments were carried out as follows: A solution of 12 g. of nicotine in 100 cc. of water was placed in each of three 5-liter flasks with oxygen and left in the light for 6 months. The solutions became a deep yellow in color and faintly acid; the oxygen was almost completely used up. After dilution of the contents of the flasks, an excess of barium carbonate was added and the product was distilled with steam. Both the distillate and the residue were examined. In the distillate were found unchanged nicotine, a small quantity of ammonia, and methylamine. From the residue there was isolated a large quantity of a product having the same composition as the oxynicotine of Auerbach and Wolfenstein (6) and of Pinner and Wolfenstein (183, 185, 186) but having a different melting point for its picrate and nicotinate. Ciamician and Silber concluded that the autooxidation of nicotine under the influence of light furnishes, besides a tar-like mass as the principal product, oxynicotine, which, by further oxidation, yields nicotinic acid and methylamine.

(b) *Oxidation by ultraviolet light.* Custis (50) found, as the result of studying the effect of light on nicotine solutions, that (i) there is a change in nicotine content when solutions of nicotine are exposed to sunlight; (ii) this change is more marked in the presence of air, a fact which indicates an oxidation process; (iii) the power of causing the change is limited to light of short wave lengths; (iv) ultraviolet light produces a change in the nicotine solutions; (v) the alkalinity of nicotine solutions does not affect the reactions due to light. Pacini and McGuigan (163) calculated that, for each nicotine molecule, the loss of toxicity, the development of a yellow color, the loss of basicity, and, finally, the development of acidity require roughly 1.9×10^{-16} gram-calories of radiant energy. The yellow color is believed by Gant (63) to be due to an oxidation product of nicotinic acid and is destroyed by further irradiation. When all of the titratable basicity of nicotine has been destroyed by irradiation there still remains unaltered nicotine in the form of its nicotinate or malonate. The acidity produced by irradiation is destroyed by further irradiation, with the production of a colorless neutral product containing no nicotine. Destruction of the pyrrolidyl ring destroys the vaso-constricting power, and the resulting products have no perceptible pharmacological action; this destruction may be avoided by excluding air, light, or both.

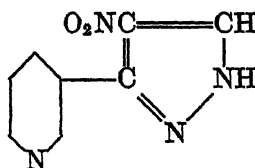
(c) *Oxidation by platinized asbestos.* The researches of Zelinskii (277) have shown that it is an easy matter to dehydrogenate a cyclohexane ring catalytically, but that a cyclopentane ring cannot be dehydrogenated in

this way. When nicotine is distilled slowly through a tube containing 2 g. of 30 per cent platinized asbestos and heated to 290–350°C., it is largely dehydrogenated to 3,2'-nicotyryne. The most favorable temperature was found to be 320°C.; isomerization did not occur, only unchanged nicotine and 3,2'-nicotyryne being obtained. After one passage of the nicotine through the tube, 73 per cent of it was transformed into 3,2'-nicotyryne; on repeating the dehydrogenation with the reaction product and with freshly platinized asbestos, an almost theoretical yield of 3,2'-nicotyryne was obtained. It thus appears that the pyrrolidyl ring can be dehydrogenated catalytically (263).

(d) *Oxidation by acids.* Nicotinic acid was first obtained by Huber (92) by oxidizing nicotine with chromic acid. Cahours and Étard (27) obtained what they called carboxypyridenic acid when nicotine was oxidized with nitric acid. Pictet and Rotschy (176) and Weidel (258) obtained nicotinic acid by oxidizing nicotine with nitric acid. In the oxidation of nicotine by nitric acid by the method of McElvain and Adams (133), Gough and King (76) found that there was formed, besides nicotinic acid, about 5 per cent of a by-product, the properties of which agreed with those of a 4-nitro-5-(3-pyridyl)pyrazole



or its tautomeric form (105).



Lund (132) determined the melting point as 220°C. and ascribed to the substance the structure of 3-nitro-5-(3-pyridyl)pyrazole, thus changing the position of the nitro group. By the oxidation of nicotine with chromic acid a well-characterized amino acid was obtained by Huber (91).

(e) *Oxidation by hydrogen peroxide.* It was shown by Pinner (183) that oxynicotine is not the primary product of the oxidation of nicotine with hydrogen peroxide, but that it is formed by condensation from an aldehyde first formed. Pinner and Wolfenstein (184) mixed nicotine with platinum sponge, then treated it with hydrogen peroxide and allowed the whole to stand for several weeks. The liquid was then evaporated at 40–50°C.

in a vacuum and extracted with alcohol. After the latter had been removed in a vacuum, the residue was allowed to remain in a desiccator until it solidified to a crystalline, deliquescent mass; the product was a base having the composition $C_{10}H_{12}N_2O$ and may be termed nicotine-*N*-oxide. Preiss (190) has shown that the action of hydrogen peroxide on nicotine solutions is strongly dependent on the hydrogen-ion concentration. The decomposition of nicotine is greatest in weakly alkaline solution, while in acid or strongly alkaline solution the substance is relatively resistant to oxidation by hydrogen peroxide. The addition of weak ammonia increases considerably the speed of oxidation of nicotine by hydrogen peroxide; the ammonia possibly exercises a catalytic action also.

(f) *Oxidation by potassium permanganate.* Laiblin (116) and Oliveri (154) obtained nicotinic acid when nicotine was oxidized by means of potassium permanganate.

(g) *Oxidation by potassium ferricyanide.* Cahours and Étard (29) prepared 3,2'-nicotyrine by oxidizing nicotine with potassium ferricyanide, but when nicotine was dissolved in dilute potash and then treated with the ferricyanide, the product was isodipyridine, $C_{10}H_{10}N_2$ (28).

(h) *Oxidation by silver oxide.* Blau (21) obtained 3,2'-nicotyrine by oxidizing nicotine with silver oxide.

2. Reduction

The reduction of nicotine by means of sodium and alcohol was first studied by Liebrecht (121) and later by Oliveri (154); both isolated a basic product which they called β,β -dipiperidyl, since they regarded nicotine as hexahydrodipyridyl. Blau (20) made a more complete investigation of the reaction and showed that Liebrecht's base was not dipiperidyl but a mixture of two amines, which he called hexahydronicotine and octahydronicotine. Pinner (179) also studied the reduction of nicotine with sodium and alcohol and reported the isolation mainly of hexahydronicotine, whereas Overhoff and Wibaut (162) obtained chiefly the octahydro compound. Windus and Marvel (265), in attempting to carry out the above synthesis, reduced nicotine with sodium and alcohol, and reduced nicotine hydrochloride with hydrogen under pressure, using the platinum oxide-platinum black catalyst of Adams and Shriner (1). They found that the reduction of nicotine by sodium and alcohol produced approximately 5 parts of hexahydronicotine to 1 part of octahydronicotine, while the catalytic reduction of the hydrochloride produced 1 part of hexahydronicotine to 3 parts of octahydronicotine.

Harlan and Hixon (81), selecting the most feasible method for the reduction of nicotine, utilized platinum oxide-platinum black and hydrogen, as used by Smith (216) on the dipyridyls, by Hamilton and Adams (79)

on the pyridine and quinoline derivatives, and by LaForge (115) for the reduction of metanictine to dihydrometanictine. Contrary to expectation, in the catalytic reduction of nicotine, rupture of the pyrrolidine ring takes place simultaneously with the hydrogenation of the pyridine ring. Complete reduction, with the absorption of 3.7 moles of hydrogen to each mole of nicotine hydrochloride, gives a mixture containing 25 per cent of hexahydronicotine hydrochloride and 75 per cent of octahydronicotine hydrochloride. If the reaction is interrupted at the point where 3 moles of hydrogen have been absorbed for each mole of nicotine hydrochloride (the theoretical amount for reduction to hexahydronicotine), the resulting mixture consists of 19 per cent hexahydronicotine hydrochloride, 57 per cent octahydronicotine hydrochloride, and 21 per cent unchanged nicotine hydrochloride. Hexahydronicotine is the sole product obtained by the reduction of nicotine with sodium amalgam (180).

3. *Effect of ultraviolet light*

Wakeham and Tracy (254) found that the exposure of nicotine to ultraviolet light produces a 70 to 75 per cent reduction of its toxicity in about 75 min., followed by a return almost to the original toxicity. Wakeham and Johnson (253) later found that no reasonable amount of ultraviolet irradiation will destroy the toxicity of nicotine, thus confirming the results of the earlier investigators.

4. *Effect of heat*

If nicotine vapor is passed over red-hot porcelain it is partly decomposed (about 20 per cent), yielding a gaseous mixture of hydrogen with paraffins and olefins, and a liquid product containing pyridine, picoline, collidine, and a new basic substance boiling at temperatures above 250°C. (28); the collidine boiled at 170°C. and showed a great tendency to form resinous polymerides (30).

Oliveri (154) obtained methylamine and a small proportion of nicotinic acid when nicotine was heated at 250–280°C. in a closed tube for about 24 hr.

5. *Reaction with elements*

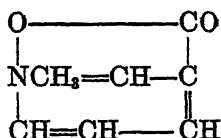
(a) *Reaction with sulfur.* Cahours and Étard (27) found that, when 5 parts of nicotine were heated to 140°C. with 1 part of sulfur, hydrogen sulfide was given off and continued to be liberated as the temperature rose to 155°C. The perfectly fluid mass assumed a chrome-green color at 160–170°C. When it was allowed to stand for some days, yellow prismatic crystals separated in abundance. These, after being purified by washing with cold alcohol and then recrystallizing from boiling alcohol, had the composition $C_{20}H_{18}N_4S$. Morton and Horvitz (143), by the dehydrogena-

tion of nicotine by sulfur in boiling toluene for 50 hr., obtained hydrogen sulfide, nicotyrine, and thiodinicotyrine.

(b) *Reaction with selenium.* When 100 parts of nicotine and 20 parts of selenium are heated together for some time at 240°C. and then briskly boiled, the condensing tube becomes filled with white lamellar crystals. When the vapor is heated nearly to redness, it is decomposed into ammonia and selenium. As soon as the crystals cease to form, the heating is discontinued, and the liquid is decanted from the undissolved selenium and distilled. Oily products pass over above 180°C., and a tarry residue is left in the retort. These products are freed from selenium by adding a solution of soda and distilling in a current of steam, the receiver being changed as soon as the distillate becomes distinctly milky. The oily substances are separated from the water by means of ether, and again distilled. In this way hydrocollidine is obtained. The other product of the action of selenium is isodipyridine; it is probable that the selenium acts on the nicotine in the same way as sulfur, removing the hydrogen as hydrogen selenide and forming isodipyridine. The hydrogen selenide then combines with unaltered nicotine, forming a hydroselenide which removes one atom of nitrogen in the form of the ammonia compound described above. When nicotine hydroselenide is subjected to dry distillation, it yields the same products as those obtained by the action of selenium on nicotine (30).

(c) *Reaction with halogens.* (i) *Iodine.* At the ordinary temperature the action of iodine on nicotine in ether or chloroform solution results in the replacement of hydrogen by iodine and the formation of periodides which are insoluble in the menstruum. The composition of these products is influenced by the relative amounts of iodine and nicotine present, as well as by the proportion of the solvent, and as these conditions are continuously varying throughout the period of reaction, various periodides of both iodonicotine hydroiodide and of nicotine hydroiodide are precipitated. Basic compounds are obtained at the same time. The results tend to support the evidence already obtained by the titration of nicotine with acids in the presence of a variety of indicators: namely, that this alkaloid normally functions as a monacid base (106).

The formation of two isomeric quaternary iodides from nicotine proves that the two nitrogen atoms of this alkaloid are both tertiary. When the isohydroxide, corresponding to the isomethiodide, is oxidized by potassium permanganate, it is converted into trigonelline (the methylbetaine of nicotinic acid).



This reaction shows that the two methiodides have the following formulas: $C_8H_4N-C_4H_7N(CH_3)_2I$ (methiodide) and $C_8H_4NCH_3I-C_4H_7NCH_3$ (isomethiodide) (174).

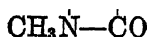
(ii) *Chlorine*. Geuthen and Hofacker (71) report the formation of a beautiful crystalline chlorine compound by the action of gaseous chlorine on nicotine hydrochloride.

(iii) *Bromine*. By the action of bromine and water on nicotine in sealed tubes at 120–150°C., Laiblin (117) obtained a crystalline compound similar to that obtained by Cahours and Étard (30), which is probably $C_{10}H_{12}Br_2N_3 \cdot HBr$. Bromonicotine is obtained by adding a mixture of 4 parts of nicotine and 5 parts of water in small quantities at a time to 12.5 g. of bromine in 7.5 cc. of water, the temperature not being allowed to rise above 50–60°C. The whole is warmed on a water bath until the oil formed is dissolved, and then 15–18 g. of water is added; on cooling a crystalline substance separates.

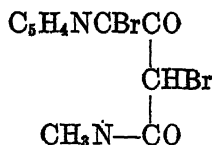
By the addition of bromine to free nicotine, large quantities of resinous products are formed. The action of bromine on a solution of nicotine in acetic or hydrobromic acid gives rise at first to an oily addition product which is undoubtedly a perbromide and from which the bromine is eliminated by treatment with sulfurous acid. In acetic acid solution the reaction quickly goes further; hydrogen bromide is formed, and the product contains the two compounds $(C_8H_4N)_2Br_2$ and $(C_8H_4N)_2Br_2O_2$; the former compound yields the well-crystallized perbromide, $(C_8H_4N)_2Br_2 \cdot HBr$, described by Huber (90) and by Cahours and Étard (29). The perbromide is obtained by adding 3.5 to 4 parts of bromine, dissolved in 4 to 5 parts of acetic acid, to a solution of nicotine in 5 to 6 parts of acetic acid. It crystallizes after 2 days in monosymmetric, chrome-red scales or flat, yellowish red needles. By treating the perbromide with ammonia the free base, dibromodihydronicotine, is obtained. Dibromodioxidihydronicotine hydrobromide is obtained by heating nicotine with aqueous hydrobromic acid and bromine in a sealed tube for 10 to 12 hr. on a water bath. It crystallizes from water in slender white needles or in colorless, lustrous, flat prisms.

The two compounds dibromocotinine and dibromoticonine are obtained by the action of bromine on nicotine; the former is obtained as the perbromide by the addition of bromine to an aqueous solution of nicotine hydrobromide. The free base is best prepared by treating the perbromide with sulfurous acid and then adding potassium carbonate. It crystallizes from dilute alcohol in beautiful colorless prisms. The hydrochloride crystallizes in needles and turns blue on heating.

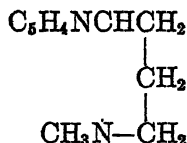
Pinner (179) assigns to dibromocotinine the constitution



and to dibromoticonine the constitution



Thence he deduces for nicotine the constitution



6. Reaction with hydrogen halides and halogen acids

With hydrogen chloride nicotine forms $\text{C}_{10}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl}$, nicotine dihydrochloride. This salt forms deliquescent crystals which are very readily soluble in water and alcohol and insoluble in ether (11). It decomposes upon the evaporation of its aqueous solution (181).

Nicotine yields well-characterized tetrachloroiodides when its solution in concentrated hydrochloric acid is added to an excess of a solution of tetrachloroiodic acid. *N,N*-Dimethylnicotine bistetrachloroiodide separates from boiling glacial acetic acid in fine yellow needles, melting at 145°C . (34). Nicotine tetrachloroiodide separates quantitatively as beautiful orange prisms when a solution of nicotine in hydrochloric acid is added to a similar solution of iodine trichloride (33).

$2\text{C}_{10}\text{H}_{14}\text{N}_2 \cdot 3\text{I}_2 \cdot 2\text{HCl}$ is obtained as beautiful bright ruby-red plates by saturating a dilute alcoholic solution of nicotine iodide with hydrochloric acid. $2\text{C}_{10}\text{H}_{14}\text{N}_2 \cdot 3\text{I}_2$ is prepared by bringing together ether solutions of nicotine and iodine and evaporating the ether; it forms beautiful ruby-red crystals (90).

Nicotine dihydroiodide, $\text{C}_{10}\text{H}_{14}\text{N}_2 \cdot 2\text{HI}$, formed by the direct action of hydrogen iodide on nicotine, crystallizes in long colorless needles melting at 195°C . When boiled with nicotine in alcoholic solution it is converted into the hydroiodide, which is a pale yellow, uncrystallizable mass (174).

Étard (60) heated 5 g. of nicotine with 5 g. of red phosphorus and 60 g.

of fuming hydrogen iodide in a sealed tube at 260–270°C. for 10 hr.; hydrogen was liberated. The crystalline contents of the tube, consisting probably of a periodide, were treated with potash, and the liberated oily bases were fractionated. The products thus obtained in small quantities were hydrocollidine and hydronicotine.

7. Reaction with acids

(a) *Benzoic acid.* Nicotine is attacked with some difficulty when boiled with benzoic acid; it yields partly the racemized original material and a non-separable mixture of benzoynornicotine and benzoymetanicotine. When nicotine is gently boiled with benzoic acid two reactions take place: (i) opening of the ring and addition of an acid residue to form the compound $C_8H_4NCH(OH)CH_2CH_2CH_2NCH_3R$ (R = acyl radical), which, by loss of water, gives the *N*-acylmetanicotine, and (ii) the normal formation of acylnornicotine (24).

(b) *d-Camphoric acid.* When nicotine and *d*-camphoric acid are heated together at 250°C., nicotine camphorate is formed (65).

(c) *Tartaric acid.* When a hot concentrated alcoholic solution of tartaric acid is added to nicotine, there soon separate white crystalline tufts of nicotine acid tartrate (55). The neutral salt of nicotine and *d*-tartaric acid, $C_{16}H_{14}N_2 \cdot C_4H_6O_6 \cdot 2H_2O$, crystallizes as needles. Its water of hydration does not disappear in a vacuum over concentrated sulfuric acid, but at 100°C. the salt gives off not only water of hydration but also nicotine (178). The acid salt of nicotine and *d*-tartaric acid forms prisms (from warm water) (171, 178).

(d) *Oxalic acid.* When the calculated quantity of oxalic acid is added to nicotine the normal oxalate is formed, but on the addition of excess oxalic acid to the normal salt the tetraoxalate is formed as nacreous lamellae. When the anhydrous salt is heated it melts at 110°C. and begins to decompose with violent evolution of gas at 113.5°C.; at 160°C. it yields the normal oxalate, and at 250°C. pure nicotine.

(e) *The lower fatty acids.* Gendre and Bary (68) made nicotine salts by the action of nicotine on acids such as formic, acetic, or lactic or by a double decomposition reaction between a nicotine salt and an alkali formate, acetate, or lactate. Gendre (66) obtained organic salts of nicotine by bringing nicotine and an organic acid such as caprylic acid into intimate contact. The salts dissolve in water and have insecticidal properties. Gendre and Bary (67) obtained British patent 446,368 (April 29, 1936) for the preparation of salts of nicotine from formic, acetic, lactic, etc. acids by the direct reaction between the acid and nicotine; these salts are commercially important on account of their insecticidal or anticyptogamic properties.

(f) *Acetic anhydride*. When nicotine is heated to 150°C. with acetic anhydride, it is converted into diacetylnicotine; this is a syrupy liquid which boils, with partial decomposition, at about 330°C. and is not affected by alkalies (61). When nicotine is heated with acetic anhydride at 170°C. for 10–12 hr., it forms acetylmecanicotine (182).

(g) *Bromoplatinic acid*. Nicotine bromoplatinate is formed by dissolving nicotine in an excess of dilute hydrobromic acid and treating the resulting solution with platinum chloride (259). Ortigosa (161) obtained nicotine platinum chloride by adding hydrochloric acid to a solution of platinum chloride in nicotine until it was neutral.

(h) *Silicotungstic acid*. North and Beal (151) obtained nicotine silicotungstate by dissolving nicotine in hydrochloric acid and then adding an excess of silicotungstic acid; the liquid, when heated to boiling and allowed to cool, gave a white crystalline product and not a salmon-colored one as described by Bertrand (16). Bertrand and Javillier (17) obtained the silicotungstate as a pinkish white salt by adding potassium silicotungstate to nicotine tartrate dissolved in water slightly acidified with hydrochloric acid (18). Heiduschka and Wolf (83) found that nicotine forms the compound $\text{SiO}_2 \cdot 12\text{WO}_3 \cdot 2\text{H}_2\text{O} \cdot 2\text{C}_{10}\text{H}_{14}\text{N}_2 \cdot x\text{H}_2\text{O}$ with silicotungstic acid and the compound $2[\text{P}(\text{W}_2\text{O}_7)_6]\text{H}_7 \cdot 3\text{C}_{10}\text{H}_{14}\text{N}_2$ with phosphotungstic acid (84). Tilici and Crestea (239) prepared the salt $2\text{C}_{10}\text{H}_{14}\text{N}_2 \cdot \text{H}_8[\text{Si}(\text{W}_2\text{O}_7)_6] \cdot x\text{H}_2\text{O}$ from nicotine and silicotungstic acid. Klyachkina, Strugatzkii, and Zilberg (108) found that nicotine combines with silicotungstic acid in the ratio of 1:2.

(i) *Nitric acid*. Nicotinic acid nitrate (pyridine-3-carboxylic acid nitrate) is prepared from nicotine and nitric acid; the salt contains one molecule of water of crystallization and melts at 190–192°C. (133).

(j) *Molybdic acid*. Nicotine ammonium molybdate is prepared by adding to 400 cc. of a 20 per cent solution of ammonium molybdate 320 cc. of a 10 per cent solution of acetic acid and 100 cc. of a 10 per cent solution of nicotine. The mixture is shaken several times and then rapidly filtered; the solid is washed successively with water, alcohol, and ether, and then dried at 48–50°C. It forms white microscopic prisms, which are insoluble in water (139).

(k) *Oleic acid, etc.* Hoyt (89) prepared nicotine oleate, coconate, and castorate by combining nicotine with dry oleic acid and with the mixed fatty acids in edible grades of coconut oil and castor oil.

(l) *Picric, picrolonic, and styphnic acids*. One mole of nicotine unites with 2 moles of picric acid to form a dipicrate, $\text{C}_{10}\text{H}_{14}\text{N}_2 \cdot 2\text{C}_6\text{H}_3\text{O}_7\text{N}_3$. This dipicrate is obtained as yellow needles, which melt at 218°C. (222); it is only slightly soluble in cold water but easily soluble in hot water (184). Nicotine picrolonate is obtained as an amorphous precipitate. Nicotine

styphnate, which is obtained as short blunt needles, melts with sublimation at 170–188°C. or at 194–195°C. (111).

(m) *β -Phenylpropionic acid*. When nicotine is boiled with β -phenylpropionic acid, the β -phenylpropionyl derivatives of nornicotine and metan nicotine are formed (24).

8. Organic derivatives

(a) *Methyl iodide*. Nicotine reacts readily with methyl iodide, producing methylnicotine, a yellow, translucent solid which dissolves in warm absolute alcohol (174, 234); its melting point is 85°C. (101). Stahlschmidt (234) prepared nicotine dimethiodide, which crystallizes in compact, light yellow prisms. Nicotine methiodide is obtained by treating nicotine with methyl iodide in mole proportions in methyl alcohol solution (174).

(b) *Ethyl iodide*. Nicotine reacts readily with ethyl iodide, yielding a yellow translucent solid which dissolves in warm absolute alcohol to form a deep brown solution (152).

(c) *Amyl iodide*. The iodide of nicotine isoamylate is prepared by heating amyl iodide and nicotine in a sealed tube on a water bath for several days (234).

(d) *Aldehydes*. (i) *Formaldehyde*. When treated with a drop of 30 per cent formaldehyde (free from formic acid) and a drop of concentrated nitric acid, nicotine gives a rose-red solution. As little as 0.5 mg. nicotine will show the reaction. Too much formaldehyde must not be added, nor must the mixture be warmed, as in either case explosive decompositions will occur (205). Reichard (199) found that, when a trace of nicotine is added to a 40 per cent solution of formaldehyde, there is immediately produced a yellow coloration which, upon the addition of a drop of concentrated sulfuric acid, changes to a color similar to that of a dilute solution of cobalt nitrate. If a trace of nicotine is mixed with one drop of 30 per cent formaldehyde, there is formed after some hours a solid residue which gives an intense rose coloration when touched with a drop of strong nitric acid; if resinous matter is present, a blood-red color appears. After some time, the color changes to green. A more permanent color is obtained when pure formic acid is used instead of formaldehyde (206).

(ii) *p-Dimethylaminobenzaldehyde*. A few crystals of *p*-dimethylaminobenzaldehyde dissolved in a drop of fuming hydrochloric acid and brought into contact with a drop of an aqueous solution of nicotine forms a pink and then a reddish-violet zone at the line of contact; finally the whole liquid becomes reddish violet. The coloration increases in intensity and is permanent for 10 to 24 hr. (247).

(e) *Pyridine and α -picoline*. In the reaction of nicotine with pyridine and α -picoline, carbon dioxide is liberated and benzylpyridinium chloride, benzylpicolinium chloride, and benzylnicotinium chloride are formed (256).

(f) *Benzyl chloride and iodide.* With benzyl chloride and nicotine, the monobenzyl- or dibenzyl-nicotinium compound is obtained, according to the proportions taken (256). A similar reaction occurs when benzyl iodide is used.

9. Inorganic derivatives

(a) *Mercury compounds.* (i) *Mercurous chloride.* Nicotine mercurous chloride is prepared by adding a solution of nicotine hydrochloride to a saturated solution of mercurous chloride until the precipitate formed no longer dissolves (23).

(ii) *Mercurous iodide.* Nicotine iodide-mercurous iodide is formed when a solution of nicotine hydrochloride is added to a saturated solution of mercurous iodide until the precipitate formed begins to persist (23).

(iii) *Mercurous cyanide.* Nicotine mercurous chloride-cyanide is obtained when a solution of nicotine hydrochloride is added to an equal volume of a saturated solution of mercurous cyanide (23).

(iv) *Mercuric oxide.* Nicotine is heated to 240°C. and mercuric oxide is added, in small quantities at a time, until the liquid becomes thick and brown. Each addition of the oxide produces an energetic reaction; water is given off and metallic mercury forms. The product of the reaction is allowed to cool and is then dissolved in dilute hydrochloric acid. The solution is filtered, and the filtrate is treated with hydrogen sulfide. The mercuric sulfide formed is filtered off, and the dark brownish black filtrate is mixed with alkali, which produces a flocculent brown precipitate having the composition $C_{10}H_{14}N_2 \cdot O_2$ or $(C_{10}H_9N_2)_3O_2$ (60).

(v) *Mercuric chloride.* Nicotine mercuric chloride, $C_{10}H_{14}N_2 \cdot HgCl_2$, is obtained by treating a solution of mercuric chloride with nicotine; it forms white crystals which are insoluble in water and ether, feebly soluble in alcohol, and decompose above 100°C. (161). The compound $C_{10}H_{14}N_2 \cdot 2HgCl_2 \cdot Hg(CN)_2$ forms prisms, which are easily soluble in hot and cold water and in alcohol without decomposition (23).

(vi) *Mercuric iodide.* Nicotine is precipitated as $C_{10}H_{14}N_2 \cdot HgI_2$ from its solution in alkali carbonate (10–15 per cent solution of potassium or sodium carbonate) by means of mercuric iodide dissolved in sodium thiosulfate (275).

(b) *Cadmium chloride.* When an alcoholic solution of nicotine hydrochloride is added to a large excess of an alcoholic solution of cadmium chloride, the compound $(C_{10}H_{14}N_2 \cdot 2HCl)_3 \cdot 7CdCl_2$ is formed; this substance crystallizes from alcohol in kidney-shaped masses. If, however, the proportions are reversed, the compound $(C_{10}H_{14}N_2 \cdot 2HCl)_2 \cdot 3CdCl_2 \cdot 2H_2O$, is obtained; it crystallizes from 50 per cent alcohol in radiant clusters of fine needles and thin plates (73).

(c) *Silver acetate and nitrate.* By the action of silver acetate on nicotine,

a basic substance is obtained which appears to be identical with a compound prepared by Cahours and Étard (38).

(d) *Sodium amide*. The action of sodium amide on nicotine in the presence of an indifferent solvent such as petrolatum or toluene leads to the formation of 2(or 6)-aminonicotine in about 18 per cent yield. After crystallization from light petroleum, it forms biaxial leaflets or plates which melt at 124–125°C. Tschitschibabin and Buchholz (245) and Tschitschibabin and Kirssanov (246) found that both isomers are produced in approximately equal amounts when nicotine is dissolved in xylene and heated with sodium or potassium amide at 135–140°C.

(e) *Gold chloride*. Varying amounts of nicotine were added to solutions of $\text{AuCl}_3 \cdot \text{HCl} \cdot 4\text{H}_2\text{O}$ (5 cc. of 0.1 per cent gold solution in 100 cc. of water), with or without added hydrochloric acid and boiling; the color of the solution was blue (32). $\text{C}_{10}\text{H}_{14}\text{N}_2 \cdot 2\text{HCl} \cdot 2\text{AuCl}_3$ was obtained; it crystallized from water as bright yellow, warty masses (181).

(f) *Potassium ferro- and ferri-cyanides*. With potassium ferrocyanide in hydrochloric acid solution nicotine yields a crystalline precipitate (42) which is characteristic enough to be used in corroborative identification tests (43). Cummings and Brown (49) prepared nicotine hydroferri-cyanide in alcoholic solution as a yellow precipitate which tends to become gummy in concentrated solution but is soluble in an excess of either reagent.

(g) *Sodium cobaltinitrite*. Sodium cobaltinitrite, when added to a neutral or slightly acidified (with acetic acid) solution of nicotine, forms greenish white needles of nicotine cobaltinitrite (167).

H. IDENTIFICATION

Kocsis and Németh (109) made use of the fluorescence of nicotine in ultraviolet light for its identification; in aqueous solutions as little as 0.1 mg. of nicotine can be thus detected. The azure-blue fluorescence is not affected by the addition of sodium or ammonium hydroxide, but it becomes a little more greenish when hydrochloric acid is added.

The most sensitive microchemical reagent is a cold saturated solution of picric acid to which 10 per cent of concentrated hydrochloric acid has been added. A yellow, amorphous precipitate, from which crystals separate in a few seconds and which is recognizable with the naked eye, is obtained with 10 μg . of nicotine. With 5 μg . of the alkaloid the precipitate is not formed; however, the same crystals generally appear, in indistinct feathery form but always of the same magnitude and arranged in groups (247).

Nicotine can be detected even in small quantities, since it is colored violet by being heated gently with a very little hydrochloric acid; the violet color becomes an intense orange on the addition of a little concentrated nitric acid (164).

From a study of the electrolytic reduction of nicotine and its salts in aqueous solution at the dropping mercury cathode, Semerano (210) established conditions for its determination; even in the presence of pyridine and ammonium bases, the alkaloid can be determined with an accuracy of 1 part in 10^7 .

Upon the dissolving of traces of nicotine in epichlorohydrin and heating to boiling, a deep red coloration is produced; about 0.00025 g. of nicotine can thus be detected (135).

The lowest concentration and minimum quantity of nicotine that can be detected by various reagents are as follows: mercuric chloride, 1:100, 0.00001 g.; potassium mercuric iodide, 1:500, 0.000002 g.; cadmium iodide, 1:1000, 0.000001 g.; cadmium bromide, 1:400, 0.000005 g.; cadmium chloride, 1:100, 0.00002 g.; zinc thiocyanate, 1:100, 0.00002 g.; cobalt thiocyanate, 1:100, 0.00002 g.; platonic chloride, 1:500, 0.000002 g.; auric chloride, 1:500, 0.000002 g.; iodine in potassium iodide, 1:100, 0.00001 g.; picric acid, 1:10000, 0.000001 g.; picrolonic acid, 1:100, 0.000005 g. (252).

I. TOXIC ACTION

Nicotine is one of the most violent poisons, hardly inferior to hydrocyanic acid in toxic power and rapidity of action, and probably acting upon all classes of animals. The absorption of nicotine takes place even in a few seconds from the tongue, the rectum, and the eyes, and somewhat more slowly from the stomach; it is also absorbed from the skin. In consequence of this rapidity of absorption, nicotine exhibits its toxic action very quickly, even after a few minutes. The elimination of nicotine proceeds through the lungs and kidneys. Although not really corrosive in its action, nicotine in more concentrated form, especially as the free alkaloid, acts as a local irritant. After the administration of fatal doses by mouth, inflammation of the mucous lining of the stomach does not usually occur, for the action of nicotine is too rapidly fatal. Moreover, following brief stimulation, nicotine exerts a central paralyzing action upon the brain and spinal cord, and finally, as a result of absorption, acts upon different organs, such as the heart, eyes, and intestinal tract. In acute nicotine poisoning death ensues from paralysis of the respiratory center, but probably action upon the heart always takes place, although this usually does not lead to death.

The symptoms of nicotine poisoning have been accurately determined by various experimenters who have taken from 1 to 4 mg. of this alkaloid. The symptoms are burning in the mouth and throat, increased secretion of saliva, headache, stupefaction, dizziness, indistinctness of vision and hearing, photophobia, parched throat, coldness of the extremities, emesis and enforced evacuation of the bowels, and accelerated and labored respiration. At first the pulse increases in frequency but later it changes, be-

coming at irregular intervals more or less frequent. After 45 min. the experimenters swooned and in one case for 2 hr. there were uninterrupted clonic convulsions, especially of the muscles of respiration, with trembling of the extremities and shaking of the whole body. In severe cases of poisoning the gastric symptoms predominate. The intense diarrhoea that appears may be bloody, resulting in death in a few hours. On the other hand, in severe cases, convulsions, delirium, and paralysis predominate and may prove fatal in 10–30 min. (7).

J. ANTIDOTE

Experiments conducted on guinea pigs and rabbits show that strychnine is not an effective antidote to nicotine poisoning; eserine is more effective, but the best antidote to nicotine is the alkaloid of *Nasturtium officinale*, two injections of the expressed juice of the plant completely counteracting the effect of a fatal dose of nicotine (0.025 g.) in the case of a rabbit; similar results were obtained with dogs (274).

III. LESSER ALKALOIDS OF TOBACCO

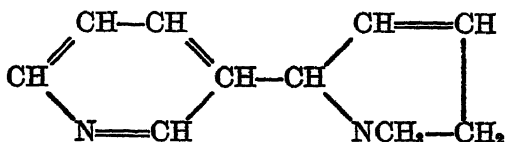
Upon the addition of a strong alkali or hydrogen peroxide, or when it was merely allowed to stand in contact with air, an aqueous tobacco extract which had previously been steam distilled and from which the nicotine had been extracted was found to yield more nicotine. This led Pinner (180) and Preiss (189) to conclude that tobacco contains a secondary alkaloid or alkaloids which, on oxidation, yield nicotine.

A. NICOTOINE, NICOTEINE, NICOTELLINE, ISONICOTEINE, AND NICOTIMINE

Noga (149) digested tobacco with water and evaporated the liquid in a vacuum. By extraction with benzene he obtained a small amount of an alkaloid which was not volatile with steam; this was fractionated into four parts, as follows: (i) nicotine, $C_8H_{11}N$, a colorless, mobile liquid possessing a strong characteristic odor, similar to that of pyridine, and having the following constants: b.p., $208^{\circ}C$.; d_4^{21} , 0.9545, n_D^{20} , 1.5105. It gave the usual alkaloid reactions; with hydrochloric, sulfuric, and picric acids, and with mercuric and platinic chlorides it formed well-crystallized salts of definite melting points. The aqueous extract remaining after the removal of the nicotine and its isomer contained two alkaloids, nicotine and nicotelline, which are not volatile with steam.

(ii) Nicotine, $C_{10}H_{12}N_2$, is a colorless liquid which is readily soluble in water and in ether; it boils at $267^{\circ}C$. and does not solidify at $-79^{\circ}C$. Its specific gravity is 1.0778 at $12.5/4^{\circ}$; n_D , 1.56021 at $14^{\circ}C$.; $[\alpha]_D$, -46.41° . The platinichloride forms yellow crystals and does not melt at $280^{\circ}C$.; the aurichloride becomes gray at $150^{\circ}C$. and melts with decom-

position at $186^{\circ}\text{C}.$; the picrate forms large transparent prisms which melt at $165^{\circ}\text{C}.$ The dimethiodide, $\text{C}_{10}\text{H}_{12}\text{N}_2 \cdot 2\text{CH}_3\text{I}$, is a yellow oil which cannot be made to crystallize. When the bromo derivative of the base is reduced by means of tin and hydrochloric acid a base is obtained which is volatile with steam but is neither nicotine nor nicotine; the formula



given to the base shows it to be closely allied to dihydronicotyrine (173) and dehydronicotine (183, 186) and explains the following facts: (a) nicotine on oxidation with nitric acid yields nicotinic acid; (b) it decolorizes aqueous permanganate in the presence of sulfuric acid; (c) its acid solutions respond to the tests for a pyrrole derivative.

(iii) Isonicotine, $\text{C}_{10}\text{H}_{12}\text{N}_2$, is a colorless, viscous, oily liquid, of rather strong and very persistent odor; b.p., $293^{\circ}\text{C}.$; d_4^{20} , 1.0984; n_D^{20} , 1.5749. Isonicotine is optically inactive, easily soluble in organic solvents, and difficultly soluble in water and in petroleum ether. It forms well-crystallized salts with hydrochloric, sulfuric, and picric acids, with mercuric, platinic, and auric chlorides, and with methyl iodide. It is oxidized to nicotinic acid, gives the pine splint reaction, and immediately decolorizes potassium permanganate in the cold.

(iv) Nicotelline, $\text{C}_{10}\text{H}_8\text{N}_2$, is only very slightly soluble in water and ether, and is best extracted from the original aqueous solution by chloroform; upon the addition of light petroleum it crystallizes from chloroform in small white needles (m.p., $147\text{--}148^{\circ}\text{C}.$; b.p., slightly above $300^{\circ}\text{C}.$). Unlike the other alkaloids of tobacco, an aqueous solution of nicotelline is neutral to litmus. It does not appear to be a pyrrole derivative and does not decolorize potassium permanganate in the presence of sulfuric acid. Its hydrochloride is easily soluble in water and its platinichloride sparingly so; its aurichloride sinters and decomposes at about $170^{\circ}\text{C}.$, and its mercurichloride melts at $200\text{--}201^{\circ}\text{C}.$ Nicotelline differs from the other alkaloids of tobacco in yielding a very sparingly soluble dichromate.

The crude nicotine obtained by Pictet and Rotschy (176) by distilling a concentrated aqueous extract of tobacco leaves with steam, contains about 0.5 per cent of its weight of an isomer, nicotine, $\text{C}_{10}\text{H}_{14}\text{N}_2$; this is a secondary base and is separated by converting it into its nitroso derivative, removing the nicotine by distilling under diminished pressure, boiling the residue with concentrated hydrochloric acid, and converting the regenerated base into its benzoyl derivative. The latter is a bright yellow oil which boils above $350^{\circ}\text{C}.$, does not solidify at $-10^{\circ}\text{C}.$, and, on hydrolysis

with concentrated hydrochloric acid, yields nicotimine as a colorless liquid boiling at 250–255°C. The base is easily soluble in water, is volatile with steam, and yields a white, crystalline, deliquescent hydrochloride. A study of its platinichloride, aurichloride, mercurichloride, and picrate shows that nicotimine is not identical with Pinner's (181) metanicotine.

B. ANABASINE

Anabasine, $C_{10}H_{13}O_2$, an isomer of nicotine, is colorless or pale yellow when pure and, like nicotine, darkens on standing; unlike nicotine, it is miscible with water in all proportions (278). Schmuck (207) stated that the alkaloid of *Nicotiana glauca* was not nicotine, as had been identified earlier by Juritz (99), but no identification was made. In 1929 Orekhov (156) isolated from *Anabasis aphylla* L. a new alkaloid which he named anabasine and in 1931 with Menshikov (158) suggested that it was probably β -pyridyl- α' -piperidine. Smith (219) examined a sample of *N. glauca* roots and found it to contain about 1 per cent of anabasine. A sample of the whole plant was later obtained from the same source and its identity confirmed; a further examination of samples of dried leaves showed that they too contained anabasine. Smith concluded that it is very doubtful that nicotine is present in this plant except possibly as a trace. Khmura (103, 104) found anabasine to the extent of 0.74 per cent in the young upper leaves of *N. glauca* and in a yield of 0.33 per cent for the entire plant.

In 1931 Ehrenstein (58) stated that Pictet's (172) nicotine had no existence. The fraction corresponding to nicotine was resolved into two fully individual alkaloids, which differed in boiling point by only a few degrees; the lower boiling alkaloid was recognized as nornicotine, and the higher boiling one as anabasine. Smith (218) isolated α -piperidyl- β -pyridine and coined for it the name neonicotine, but Ehrenstein (58) suggested that the substance was probably the *dl*-form of anabasine.

Späth and Mamoli (229) synthesized anabasine by condensing *l*-benzoyl-piperidone with ethyl nicotinate and submitting the product to the action of hydrochloric acid at 130°C., Δ^2 -2-(3'-pyridyl)tetrahydropyridine (anabasine) was thus obtained. This substance, on reduction, gave *dl*-anabasine, which was deracemized by Späth and Keszler (225) by the use of *l*-6,6'-dinitro-2,2'-diphenic acid to separate *l*-anabasine, $[\alpha]_D^{18}$, -82.45° ; to isolate *d*-anabasine ($[\alpha]_D^{18}$, $+62.11^\circ$) from the mother liquor, *d*-6,6'-dinitro-2,2'-diphenic acid was used.

The boiling point of anabasine has been reported as follows: 104–105°C. at 2 mm. (158); 137.5–138.5°C. at 10.5 mm. (58); 145–146°C. at 15 mm. (156); 155°C. at 19 mm. (58); 276°C. (158); 277.5–280°C. (218); 280–281°C. (72); 280–282°C. (217); 280.9°C. (147); 281°C. (219).

The following values have been given for the density of anabasine: d_4^{20} , 1.044 (156); d_{20}^{20} , 1.0455 (158), 1.046–1.048 (278), 1.0481 (147).

The specific rotation of anabasine has been reported as follows: $[\alpha]_D^{20}$, -9.1° (219); $-47^\circ 21'$, -59.66° (202); -82.2° (158); $[\alpha]_D^{15}$, -81.7° , 20.6° (in two equivalents of hydrochloric acid, $c = 1.75$) (227). According to Orekhov (156), Norkina and Narkuziev (150), and Orekhov and Menshikov (158), the specific rotation of anabasine varies with the solvent and racemization is apt to occur during extraction.

Gorbachev (75) determined the vapor pressure of anabasine by deter-

TABLE 34
Vapor pressure of anabasine

TEMPERATURE	PRESSURE OBSERVED	TEMPERATURE	PRESSURE OBSERVED
$^\circ\text{C.}$	<i>mm.</i>	$^\circ\text{C.}$	<i>mm.</i>
79.0	2.5	218.6	211.5
100.0	6.0	280.6	756.8
137.1	31.6	280.9	760.0
184.2	85.4	282.4	776.1

mining the boiling points in a manostat; the experimental results were quite in agreement with the Kistyakovskii equation:

$$\log P = \log T - (2586.6/T) = 1.0143$$

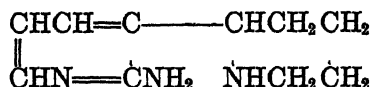
Nelson (147) obtained the vapor pressure within an accuracy of about 5 per cent up to 100°C. and to better than 0.10 per cent for the remainder of the temperature range investigated by the empirical equation

$$\log P_{\text{mm.}} = 7.2423 - (2416.14/T_x)$$

Optically active anabasine sulfate has been found to undergo racemization when heated in a sealed tube (137). It was observed that, after a dilute solution of the sulfate had been heated in a sealed tube at 200°C. for 70 hr. and the anabasine had been freed, the rotation changed to $[\alpha]_D^{20} = -25^\circ$ from presumably $[\alpha]_D^{20} = 82.2^\circ$, while after heating for 120 hr., the value of $[\alpha]_D^{20}$ was -3.0° . About 10 months after a rotation of -59.66° was found for anabasine, another sample was purified; this sample showed a rotation of $[\alpha]_D^{20} = -51.93^\circ$, thereby indicating autoracemization at room temperature.

On hydrogenation in the presence of platonic oxide, anabasine yields a mixture of bases including *l*-2,3-dipiperidyl (m.p., $66\text{--}68^\circ\text{C.}$; b.p., $113\text{--}114^\circ\text{C.}$ at 5 mm.; $[\alpha]_D^{20}$, -5.0° (alcohol) (157)). When anabasine is heated

with silver acetate or refluxed with zinc dust, six atoms of hydrogen are lost; a bipyridyl is formed (158). Treatment of anabasine in boiling xylene with sodium amide gives about 5 per cent of aminoanabasine,



which melts at 111°C. (138).

When anabasine is heated with formaldehyde and formic acid, *N*-methyl-anabasine is obtained readily and in good yield (159); it can also be prepared by refluxing anabasine with 40 per cent formaldehyde or by treating the alkaloid with magnesium and methyl iodide in absolute ether (160). Anabasine, when treated with potassium permanganate, gives nicotinic acid in good yield (158).

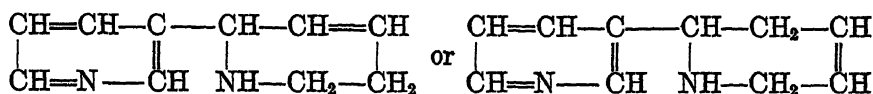
Sokolov (221) has shown that fluorosilicic acid is an excellent reagent for the distinguishing of nicotine from anabasine; the latter is precipitated as anabasine fluorosilicate in the presence of nicotine.

Anabasine stimulates and finally paralyzes the cervical sympathetic ganglia and in a concentration of 1:1,000,000 causes a flow of adrenaline from the isolated adrenal gland. The activity ratio of nicotine to anabasine for constriction of the vessels of a rabbit's ear is 1.5:1, for the rise of arterial pressure in a cat it is 2.55:1; and for respiratory stimulation it is 4:1 (5). The results of Sargin's (202) experiments indicate that anabasine is very similar to nicotine in its action; it changes the blood pressure and affects the intestines and uterus in much the same way as does nicotine.

C. *L*-ANATABINE, C₁₀H₁₂O₂

Späth and Keszler (226), in a continuation of the study of the concomitant bases of tobacco undertaken by Späth and Zajic (233), examined a fraction boiling at 120–140°C. at 1 mm. This was further fractionated and the products were converted into dipicrates, one of which had a melting point of 195–196°C. and yielded a base having a specific rotation, $[\alpha]_D$, of -141° . This substance, on purification, yielded a base which had the following properties: b.p., 145–146°C. at 10 mm.; $[\alpha]_D^{17}$, -177.8° ; d_4^{19} , 1.091; n_D^{20} , 1.5676. The name anatabine was suggested for this substance to indicate its origin from tobacco. On dehydrogenation with palladium sponge under mild conditions, *l*-anatabine yields 2,3'-dipyridyl. The supposition that anatabine contains a non-hydrogenated and a partially hydrogenated pyridine ring was confirmed by its catalytic hydrogenation to *l*-anabasine by means of palladium sponge in acetic acid. The optical rotation of *l*-anatabine and the fact that in its benzylation no opening of the ring and addition of water occur, exclude positions adjacent

to the secondary nitrogen atom for the double bond of the partly hydrogenated ring and leave the possible structure as:



Reactions take place which show the possibility of both formulas or either one. Anatabine shows certain similarities to the tobacco base which Ehrenstein (58) erroneously thought was *l*-anabasine.

D. NICOTYRINE

By steam distilling finely ground Brazil tobacco, Wenusch (261) obtained nicotyrine. It is not clear whether this substance preëxists in the plant or is produced in the fermentation process. If it is purely a fermentation product, it is derived from nicotine, because the Brazil tobacco is free from reducing substances.

E. *l*-NORNICOTINE

l-Nornicotine was found by Smith (220) in *Nicotiana sylvestris* to the extent of about 95 per cent of the total alkaloid content. After the cautious oxidation of *l*-nicotine with potassium permanganate and the removal of the more strongly basic portions from the product by fractional extraction with hydrochloric acid, followed by distillation with steam *in vacuo* (whereby nicotine is volatilized), the residue is transformed into the dipicrate, from which, after repeated crystallization, *l*-nornicotine is isolated. Its specific rotation, $[\alpha]_D^{20} = -76.1^\circ$, is raised to -83.2° after further purification through the perchlorate. When *l*-nicotine is treated with silver oxide and the product, dissolved in ether, is treated with aqueous sodium chloride containing hydrochloric acid, there is obtained a very strongly basic fraction from which *l*-nornicotine ($[\alpha]_D^{20}, -88.8^\circ$) is isolated. Its specific gravity is 1.07, and its melting point is $191-192^\circ\text{C}$. (230, 233).

IV. ALKALOIDS OF TOBACCO SMOKE

The following alkaloids, apart from the normal alkaloidal constituents of tobacco, have been isolated by Wenusch and Schoeller (quoted by Späth, Wenusch, and Zajic (237)) from tobacco smoke: (1) bases volatile with steam,—myosmine, obeline, α -socratine, β -socratine, and γ -socratine; (2) bases not volatile with steam,—anodmine, lathreine, and lohitam. These bases are obtainable only in minute quantities, and only one of them, myosmine, $\text{C}_8\text{H}_{10}\text{N}_2$, has been fully investigated.

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SYMPOSIUM ON THE MOLECULAR STRUCTURE OF FATS AND OILS¹

INTRODUCTION TO THE SYMPOSIUM

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The enthusiasm which greeted the suggestion of organizing a symposium on the Molecular Structure of Fats and Oils for the American Chemical Society meeting in St. Louis (April, 1941) was gratifying and contagious. Without exception, every chemist with experience in the study of lipids who was consulted regarding the program expressed hearty approval of the proposal. The attendance and discussions at the meeting gave further evidence of the widespread interest and timeliness of the subject.

The fats and oils are far more complex than is generally realized, not only with respect to the multiplicity and variation in the types of materials present but also in the nature of the dominant group of compounds, the fatty acid glycerides. It seems necessary to emphasize repeatedly the fact that our knowledge of the molecular structure of the fats and oils is extremely fragmentary. The elementary concept of "glycerol and three fatty acids" is still held too widely as an adequate picture. To the chemist, who is in a position to appreciate the great importance of knowing the nature of the fundamental molecular units in any material that is used extensively, the study of fats and related lipid materials presents a worthy and exciting challenge.

The quantities of oils and fats that enter into commercial usage are impressive. We produce annually in the United States approximately 1.5 billion pounds each of butter and lard and nearly 2 billion pounds of cottonseed oil. We consume nearly half a billion pounds of oil in the highly technical paint and varnish industry, and we have a factory utilization of approximately 5 billion pounds of fats per year. Yet we know relatively little about many phases of their molecular structure or the chemical steps involved in the synthesis, breakdown, and reconstruction of fats in

¹ This Symposium was held under the auspices of the Division of Biological Chemistry and the Division of Agricultural and Food Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Missouri, April 7-11, 1941.

either animals or plants. It seems reasonable to suggest, then, that industry, agriculture, and even medicine are likely to reap a good return from continued fundamental research.

A special note of indebtedness for assistance in making the symposium possible is due the officers of the participating Divisions: Division of Biological Chemistry, H. O. Calvery, *Chairman*, and E. Brand, *Secretary*; Division of Agricultural and Food Chemistry, G. A. Fitzgerald, *Chairman*, and C. R. Fellers, *Secretary*. R. C. Newton and A. S. Richardson, who served as presiding officers, were also helpful in arranging many of the details of the program.

COMPOSITION AND STRUCTURAL CHARACTERISTICS OF GLYCERIDES IN RELATION TO CLASSIFICATION AND ENVIRONMENT¹

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I. INTRODUCTION

The steadily increasing volume of analyses of natural fats during the past two decades has emphasized two things: (a) that plants and animals classed together by biologists on the basis of their structural peculiarities frequently elaborate similar kinds and proportions of fatty acids combined as triglycerides, and (b) that the most intricate structures and most complex fatty acid mixtures are found in fats of the simplest forms of plant and animal life. A gradual simplification is observed both in structure and number of component acids on ascending the evolutionary scale of development. Hilditch (97) has commented on these facts and has suggested that biochemical changes in fats have accompanied evolutionary development (126). The available information, much of which is either derived or inspired from Hilditch's laboratory, has recently been assembled in a monograph (100) which clearly expresses the various interrelationships. This monograph should be consulted for the extensive details of composition of some one hundred fats of aquatic origin, four hundred and twenty from plant species, and about eighty from land animals. It is possible to give here only a brief survey of the vast amount of available information.

A complete knowledge of the composition of natural fats in terms of

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constituent acids and glycerides is desirable for the intelligent use of the many available products (96). Detailed quantitative information regarding the individual component acids provides a means of sorting the fats for most purposes. Such data are not always adequate, however, for the correct prediction of the physical or chemical behavior of the fat. The basis for the physical dissimilarity of cocoa butter and mutton tallow, despite their similar fatty acid composition, lies in the nature of the glycerides found in the two fats (55, 58, 120, 121). Other examples demonstrating physical anomalies may be selected in comparing partially hydrogenated vegetable oils with naturally occurring glyceride mixtures of nearly identical acid composition (112). Recently, in connection with studies on cottonseed oil, Hilditch and Maddison (128) again pointed out that the analyses of component acids may be interpreted in several ways in deducing the probable component glycerides.

In most cases, the qualitative identification and quantitative measurement of fatty acid composition is possible. However, the close similarities in physical properties of the glycerides makes their separation and quantitative analysis exceedingly difficult. Only in the case of relatively few fats have the component glycerides been studied, and even then the compositions have been expressed for the most part in general terms rather than as specific configurations. A beginning has been made towards the understanding of structural peculiarities of natural fats and of changes effected during their hydrogenation to successive stages of saturation. Interestingly enough, the study of partially hydrogenated products has been a major aid in the elucidation of the structure of glycerides originally present in an oil. Much more work is needed, however, to provide a completely satisfactory picture of either the original structures present in natural fats or those which arise from some treatment, such as hydrogenation, selective adsorption, selective extraction, molecular distillation, or fractional crystallization.

While our present knowledge of the exact constitution of glycerides is far from complete, certain broad generalizations have emerged. The construction of glycerides seems to take place along lines which are similar for natural fats from a wide variety of plant or animal species. Glyceride molecules are fashioned largely independently of either the particular acids present or any biological relationship. A maximum formation of mixed triglycerides with several fatty acids, rather than simple triglycerides containing only one acid, is the regular observation.

II. COMPONENT ACIDS OF NATURAL FATS

A study of the organization of animal or vegetable fats, which are in the main triglycerides, is logically approached by referring first to the

qualitative and quantitative nature of the component fatty acids. Comparatively simple and satisfactory methods are available for the quantitative determination of fatty acids by the ester fractionation procedure (8, 99, 195, 269). A mixture of acids obtained from a given fat is first separated into two groups on the basis of the solubility of their lead salts in alcohol (101, 141, 263). The soluble portion contains most of the unsaturated acids, together with saturated acids of lower molecular weight (C_8 to C_{12}) and some myristic and palmitic acids; the insoluble portion is made up mainly of saturated acids but also has some oleic acid and, if they were present in the original sample, elaidic, isoöleic (e. g., petroselinic acid or partially hydrogenated linoleic acid), eleostearic, erucic, or hydroxy acids. A similar separation may be effected by the fractional crystallization of the original acid mixtures (36). Each group of acids is then converted to neutral methyl esters for fractional distillation. From qualitative identifications of the acids present and analytical data such as saponification equivalent, iodine value, and thiocyanogen number for the various fractions, the composition of each fraction and eventually of the entire mixture may be readily calculated (92, 97, 197).

A. Fats of aquatic origin

An exceptional variety of acids, predominantly unsaturated, distinguishes aquatic fats from those of higher plant and animal species. Only 15 to 20 per cent of saturated acids are found; these are mainly palmitic acid and small amounts of stearic, myristic, lauric, and caprylic acids (223). The unsaturated acids, on the other hand, vary in amount and extent of unsaturation from C_{16} to C_{24} . Not all of the unsaturated acids have been identified with certainty, and analyses are customarily expressed in terms of the mean unsaturation of groups of unsaturated acids of the same carbon content. For example, $C_{18(-3H)}$ is used to indicate a mixture of C_{18} acids whose mean unsaturation corresponds to 1.5 double bonds. The determination of the fatty acid compositions of such complex fats has not been accomplished within the most desired limits of accuracy (92). Nevertheless, the differences which appear for the compositions of fats from different species are in many cases three to ten times the possible experimental error, making it decidedly unlikely that such observations are fortuitous.

For example, gross differences exist between the fats of sea- and fresh-water algae or plankton. The sea-water or marine fats have, in general, the more complex mixtures with high proportions of C_{18} , C_{20} , and C_{22} unsaturated acids. Fats of fresh-water algae contain larger proportions of C_{16} and C_{18} unsaturated acids, with little C_{20} and practically no C_{22} acids (90, 103, 205). In both cases there has been found an extremely high average unsaturation for acids of the C_{20} and C_{22} series, indicating the

presence of three to six ethylenic linkages. The compositions of the fats of many larger forms of water life show these same general features.

The fats of some species show special features in composition, and in many fish the liver serves apparently as a main depot for fat storage, in contrast to the situation in higher animal species. In several cases (some Elasmobranch families* and the *Physeteridae*, or sperm whales) fatty acids are linked with glycerol as ethers rather than esters or are in combination with other alcohols than glycerol (6, 107, 124, 125, 206, 258, 259, 270). The unsaturation of the acids is usually low.

The dolphin and porpoise family (*Delphinidae*) presents one of the most interesting of extremes in fatty acid composition. The jaw, head, and blubber fats of this family of marine animals have substantial proportions (30 to 45 per cent) of the branched-chain, uneven-numbered carbon chain acid, isovaleric acid (49, 81, 203, 271). This acid is absent from the liver, lungs, and heart (203).

Considerable study has been given whale blubber fat because of its technical significance (7, 152, 262). A higher degree of unsaturation in Arctic whale oil, as compared with Antarctic oils, has been reported. Whale oil is typical of the marine type of fats in composition, with sizeable quantities of C_{20} and C_{22} unsaturated acids.

Salmon body fats show an interesting progressive change from the fresh-water type of component fatty acid mixtures to the marine type, as the fish develop from purely fresh-water animals (two to three years old) into adult fish and swim out to sea. Lovern (204) has studied these changes at several stages in the salmon's life cycle.

B. Fats of land animals

A decided simplification in fatty acid composition accompanies the increased specialization of higher orders in the scale of evolutionary development. The change in the type of fat is not an abrupt one. Amphibia and reptiles retain considerable proportions of unsaturated acids (83, 132, 135, 181, 183). There are, however, notable decreases in the percentages of C_{16} , C_{20} , and C_{22} unsaturated acids from those found in aquatic fats. These decreases are compensated chiefly by increases in C_{18} acids which are somewhat more unsaturated than oleic acid.

A single detailed analysis (208) of the fat from an invertebrate, the earthworm, indicates the desirability of studying other invertebrates. Acids ranging from C_{10} to C_{22} with 70 per cent of highly unsaturated acids were found.

A few quantitative determinations of the composition of bird depot fats (114, 150) have been made. Only small amounts of C_{20} and C_{22} acids are found in hen fats and, in general, the composition is more closely related

to that of fats of the higher species of land animals than to aquatic fats. A characteristic feature is the occurrence of 27 to 30 per cent (molar) of palmitic acid with only small amounts of stearic acid (*ca.* 5 per cent).

It is important to bear in mind that animal depot fats may arise either by synthesis from non-lipid sources in the diet or, when rations contain relatively large proportions of fat, by direct assimilation of the ingested fatty acids. The extent to which either or both processes operate in the formation of animal depot fats determines to a considerable extent the degree to which such fat is characteristic of the animal.

Thus the composition of the body fat of sea birds, the food of which is mainly fish, probably reflects the nature of their diet rather than a relation to biological species (187). Lovern (207) has analyzed the depot fats of several sea birds and has found a considerable increase in the proportions of C_{20} and C_{22} unsaturated acids at the expense of C_{18} unsaturated acids and palmitic acid. These fats resemble the marine type of aquatic fats. Lovern suggests two possible explanations for this divergence from the broad rule that the type of fat may be correlated with phylogenetic relationships: (a) that sea birds have no specific requirements and any type of depot fat will serve equally well, or (b) that their specific requirements have been produced or modified to suit the animal's normal diet.

Most quantitative studies of the effect of dietary and other conditions on animal body fat have been made on the rat and the pig. In order to evaluate the extent to which food fat influences the body fat, it is necessary to know the composition of fat formed exclusively, or almost entirely, from dietary sources other than fat. On low fat diets, these animals synthesize body fats which have several points of interest (16, 18, 25, 36, 48, 60, 73, 74, 75, 122, 146, 183, 196, 199, 200, 240). As is characteristic of fats of most higher animals, the fats of both the rat and the pig contain 25 to 30 per cent of palmitic acid. Stearic acid is found in small proportions in rat fat (*ca.* 5 per cent) but to a somewhat greater extent in pig fats (*ca.* 10 to 15 per cent). While oleic acid is predominant among the unsaturated acids in both cases (45 to 50 per cent), the quantity of the C_{18} monounsaturated acid,—palmitoleic acid ($\Delta^9,^{10}$ -hexadecenoic acid),—is relatively high in rat fats formed from non-fatty sources. Small amounts (*ca.* 1 per cent) of more highly unsaturated acids are found. The effect of including fat in the diet has been shown to result in considerable alterations in the composition of the fat without any apparent disturbance to the animals, who seem to be quite capable of tolerating well the gross changes in type of body fat. In the rat, the direct deposition (233, 234) of food fatty acids always results in a considerable reduction in the proportion of palmitoleic acid (to *ca.* 5 per cent), no change in stearic acid, and complete changes for those acids present only in small amounts in the

TABLE 1
Fatty acid composition of various animal fats (in weight percentages)

ANIMAL	SATURATED ACIDS				UNSATURATED ACIDS				
	C ₁₄	C ₁₆	C ₁₈	C ₂₀ (-2H)	C ₁₈		C ₂₀ (-2H)	C ₂₂	C ₂₄
					Oleic	Linoleic			
Liver oils:									
Cod (91).....	2	14	1	2	26	(-3.3H)	25	(-5.5H)	20 (-7.4H)
Halibut (202).....	3.9	15.1	0.5	18.7	34.4	(-2.0H)	13.8	(-5.5H)	13.6(-7.6H)
Fresh-water fish (202):									
Pike.....	4.7	13.2	0.5	20.8	38.4	(-2.8H)	15.3	(-7.5H)	6.3(-7.5H)
Perch.....	3.5	12.5	2.0	1.1	40.5	(-3.2H)	13.8	(-6.8H)	7.1(-9.2H)
Whale (152).....	6.3	18.2	2.4	3.7	38.4	(-2.6H)	11.4	(-5.6H)	6.3(-9.0H)
Salmon (204):									
Young.....	3.4 ^a	17.7	3.3	3.1	30.0	(-3.8H)	12.9	(-8.3H)	9.9(-10.2H)
Adult.....	2.7	14.3	5.0	0.7	32.2	(-3.2H)	19.4	(-6.7H)	17.8(-9.4H)
Frog (181).....	4	11	3	15	52	(-2.5H)	15	(-6H)	
Lizard (135).....	4	29	10	12	40	(-2.7H)	5	(-5.5H)	
Earthworm (208).....	12 ^a	8	12	7 ^d	20	(-3.9H)	21	(-6.8H)	18 (-?H) ^e
Chicken (114).....	0.1	25.6	7.0	7.0	38.4	21.3	0.6	(-8H)	
Rat (196):									
Low fat diet.....	3.1	26.7	4.0	0.9	47.2	2.2	0.3	(-8H)	
5 % fat diet.....	1.8	24.4	4.4	0.3	44.3	18.0	1.4	(-8H)	
High corn oil diet.....	0.7	12.2	3.1	6.9	44.8	32.3			
High coconut oil diet.....	44.5 ^f	19.9	4.0	2.0 ^g	23.8	1.4			
Pig (back fat):									
Low fat diet (122).....	0.8	25.9	12.2	0.2	48.1	7.8	3.0	(-8H)	
Low fat diet (74):									
Alone.....	1.7	25.5	13.7		50.2	8.9			
+4% cottonseed oil.....	1.1	25.0	21.1		39.5	13.3			
+12% cottonseed oil.....	1.1	13.8	26.5		31.8	26.8			

Beef tallow (123).....	3.3	24.9	24.9	0.4	2.4	41.8	1.8	0.5(-8H)	
Mutton tallow (58).....	4.6	24.6	30.5			36.0	4.3		
Goat (67).....	5.6 ^a	25.5	28.1			38.4		2.4(-8H)	
Organ glycerides:									
Beef liver (146).....	1.4	32.5	5.0	1.5	11.7	40.3(-3.0H)		7.4(-6.9H)	
Pig liver (146).....	0.1	25.8	10.5		9.2	44.1(-2.4H)		9.2(-6.8H)	
Beef heart (182).....		22	20		12	45			1.1(-6.8H)
Beef adrenal phospholipids (13).....	1.2	23.8	13.1 ⁱ			40.2		22.2(-8H)	

^a Including 0.7 per cent C₁₂.^b (-2.3H).^c Including 6 per cent C₁₂.^d (-2.7H).^e Including 8 per cent C₁₄.^f Including 27.2 per cent C₁₂.^g Including 0.6 per cent C₁₂.^h Including 3.5 per cent C₁₂.ⁱ Including 2.0 per cent C₂₀.

"synthetic" fat, such as saturated acids of low molecular weight, like lauric and myristic, or highly unsaturated acids such as linoleic, linolenic, or arachidonic (48, 196). Pig depot fats respond somewhat differently to food fatty acids.

For the next higher forms of land animals, the recorded data are indeed meagre, but they show again some additional simplification in fatty acid composition. In beef and mutton tallow, for example, three acids predominate,—palmitic, stearic, and oleic; they are present in the proportions 25 to 30, 20 to 25, and 40 to 45, respectively (15). It is of interest that little perceptible effect on the body fat of steers was produced by feeding liberal allowances of oils containing highly unsaturated acids (255).

The progressive changes in composition observed in passing from the simpler to the more complex biological species are indicated in table 1.

C. Vegetable fats

For the most part, the component acids of vegetable fats are not so complex as many animal fats (table 2). The acids which predominate are palmitic, oleic, and linoleic. Certain distinctive acids are found in special cases, usually only in the seed fats from the same or related plant families. The relationship between the composition of the seed fat and the botanical grouping is considered here for several component fatty acids (*cf.* Hilditch (100) for a more detailed discussion). A few representative analyses of common and unusual vegetable fats (out of hundreds available) are shown in table 2.

It is apparent that the highest amounts of linolenic acid are found in seeds from large trees (pine (1), walnut) and certain herbaceous plants (perilla, linseed, hemp), together with large amounts of linoleic acid and some oleic acid. Several families other than the ones represented by these trees and shrubs have been found to contain linolenic acid but in variable amounts in different species. Thus blackberry (193), rubber (86, 158), and *Stillingia* (170) seed fats contain from 20 to 25 per cent of linolenic acid. In related species of these families (*Rosaceae* and *Euphorbiaceae*), however, linoleic and linolenic acids are largely replaced by exceptional acids such as licanic acid (oiticica nut, 74 to 80 per cent (176, 209, 216)), eleostearic acid (tung seed, 74 to 95 per cent (176, 201, 215, 217, 218, 241, 263)), or ricinoleic acid (castor oil, 88 to 92 per cent (224, 244)). Linoleic acid is the predominant acid in the above group of "drying" oils. It is also found extensively in the absence of linolenic acid in a series of oils which are either "semi-" or "non-drying" (*cf.* table 2 and references 19, 163-166, 168, 228, 237).

In all these cases, the saturated acids rarely form more than 10 per cent of the total component acids. Several plant families, e.g., *Malvaceae*

(cottonseed), *Solanaceae* (tobacco, tomato, etc.), *Anacardiaceae* (cashew, pistachio, etc.), and *Bombaceae* (kapok (169)) have characteristically a

TABLE 2
Fatty acid composition of several vegetable fats (in weight percentages)

VEGETABLE FAT	SATURATED ACIDS			UNSATURATED ACIDS		
	Myristic	Palmitic	Stearic	Oleic	Linoleic	Linolenic
<i>Pericarp fats:</i>						
Palm oil (61).....	2.7	42.5	3.4	40.9	10.5	
Piqui-a (143).....	1.5	45.1	1.8	49.6	2.0	
Stillingia tallow (140).....	1.4	48.4	0.9	46.0	3.3	
Olive oil (153).....	0.5	10.0	3.4*	77.5	8.6	
<i>Seed (kernel) fats:</i>						
Perilla (174).....		6.7		10.7	33.6	49.0
Linseed (113).....	0.2	5.4	4.1*	9.6	42.6	38.1
Stillingia seed (170).....		4.7	1.9	8.1	59.4	25.9
Cedar nut (156).....				32.5-35.8	31.1-34.2	16.6-27.8
Hemp (86).....		5.8	1.7	6.7	68.8	15.9
Hemp (155).....		24.1	11.7	45.7	18.5	
Walnut (86).....		7.0	1.1	19.1	65.9	6.9
Grape (167).....		6.3	3.1*	33.5	54.6	2.4
Safflower (162).....		4.2	2.0*	26.3	67.4	0.1
Horse chestnut (177).....		4.4	3.6	67.1	22.7	2.2
Thorn apple (108).....		3.3	5.1	17.1	74.5	
Poppy (71).....		4.8	2.9	30.1	62.2	
Sunflower (21).....		3.5	2.9	34.1	58.5	
Sesame (110).....		9.1	5.1*	45.4	40.4	
Tobacco (231).....		3.3	5.1	17.1	74.5	
Tomato (89).....		5.7	10.7*	45.4	38.2	
Grapefruit (161).....		20.3	7.6	20.7	51.4	
Cottonseed (142).....	2.0	19.6	3.4	24.6	50.4	
Coffee (235).....		28.2	12.7	17.3	35.8	
Brazil nut (236).....		14.3	2.7	58.3	22.8	
Cashew nut (155).....		4.1	6.0	68.2	21.7	
Pistachio nut (65).....	0.6	8.2	1.6	69.6	20.0	
Mowrah butter (109).....	0.6	7.6	1.4*	83.3	7.4	
Margosa (137).....		14.9	15.7*	61.9	7.5	
Shea butter (84).....		5.7	41.0	49.0	4.3	
Phulwara butter (42).....		56.6	3.6	36.0	3.8	
Cocoa butter (151).....		24.4	35.4	38.1	2.1	
Borneo tallow (139).....	1.4	21.5	39.0	38.1		

* Indicates the inclusion of a small amount (0.5 per cent) of arachidic acid.

slightly higher saturated acid content (15 to 25 per cent, largely palmitic acid), and in this respect they resemble seed fats of grains such as barley (252), rice (157), rye (245), wheat (159, 249), and corn (20, 196). The

appearance of stearic acid as a major component acid is observed in some tropical families for which the seed fats of several genera have been thoroughly studied (*cf.* table 2 and reference 145). It is interesting to note that only small amounts of linoleic acid are present when the content of stearic acid is high. While this might be suggestive of some kind of biological interrelation between the C_{18} acids or their immediate precursors, Hilditch (100) prefers to regard the presence of such large amounts of stearic acid as a specific characteristic of the seed fat in a given plant family.

Saturated acids other than palmitic and stearic occur in the seed fats of only a few specific families. Lauric acid is the only major saturated acid found in the *Lauraceae* (cinnamon (226), laurel oil (56, 57)); myristic acid similarly characterizes the *Myristicaceae* (nutmeg (56, 57)). The two acids, lauric and myristic, occur together in large proportions as the chief saturated acids in several other families (43, 57, 87, 243). Both the seed and fruit coat fats of the *Palmae* are characterized by their high amounts of lauric acid (45 to 50 per cent) along with substantial amounts of myristic acid (15 to 20 per cent) and 5 to 10 per cent each of caprylic, capric, palmitic, and stearic acids (e.g., coconut oil (9, 27, 55, 196, 222, 253) and palm oil (10, 55, 61, 102, 118, 119, 171)).

Saturated acids of higher molecular weight,—arachidic, behenic, and lignoceric,—are present almost exclusively in the seed fats of *Leguminosae* and *Sapindaceae*, although not all the species studied have been alike in this respect. Arachidic acid occurs as 20 per cent of the mixed acids of kusum oil (66), and there are 5 to 7 per cent of C_{20-24} saturated acids in peanut oil (110, 113, 154, 160, 172, 195). Soybean oil (68) has only minor amounts of these acids.

The occurrence of unusual unsaturated acids in particular plant families has been referred to above in connection with linolenic, licanic, and eleostearic acids. Several other unsaturated acids characterize different plant families. Petroselinic ($\Delta^{6,7}$ -octadecenoic) acid, for example, is common to the seed fats of umbelliferous plants such as parsley (115, 268), parsnip, fennel, celery, carrot, caraway, coriander (50), and others (117, 225, 242, 261). Similarly, erucic acid is elaborated in most Cruciferous plants (e.g., rape seed (134, 144, 246), wallflower seed (116), and jamba seed (178, 247)) and in the seed fats of the nasturtium (80, 129, 248). The cyclopenteno acids,—hydnocarpic, chaulmoogric, and gorlic,—have been found only in the seed fats of the *Flacourtiaceae* (54).

III. COMPONENT GLYCERIDES OF NATURAL FATS

The structure of glycerides found in natural fats presents a complex problem in isomerism. In no fat from a natural source is there just one

fatty acid. Even the simplest fats have several different acids which vary in length of carbon chain, degree of unsaturation, and configuration, and there is the possibility of locating each fatty acid at any one or all of the three carbon atoms of glycerol. The possibility of combination with the optical antipodes of glycerol to produce optically active glycerides adds an additional complication which, perhaps fortunately, has not yet been found in natural fats.

The number of possible glycerides increases geometrically with each additional fatty acid. If n = the number of component acids, then the theoretical number of combinations which would result from a maximum even distribution of the acids in the three positions of glycerol is n^3 . The actual number of molecules distinguishable chemically is $\frac{1}{2}(n^3 + n^2)$.

TABLE 3

The numerical relationship between component fatty acids and glycerides

NUMBER OF FATTY ACIDS (n)	THEORETICAL COMBINATIONS (n^3)	NUMBER OF GLYCERIDES $\frac{1}{2}(n^3 + n^2)$
1	1	1
2	8	6
3	27	18
4	64	40
5	125	75
6	216	126
7	343	196
8	512	288
9	729	405
10	1000	550

Thus in the case of just two fatty acids, O and P, there will be eight (2^3) theoretically possible combinations with the trihydric glyceryl radical, CH_2CHCH_2 (G), as follows:

(i) G (O O O)	(v) G (O P P)
(ii) G (O O P)	(vi) G (P O P)
(iii) G (O P O)	(vii) G (P P O)
(iv) G (P O O)	(viii) G (P P P)

It will be observed that (ii) and (iv) and (v) and (vii) are identical chemical molecules, because the end carbon atoms of glycerol are identical. Therefore, the actual number of individual glycerides which may be obtained by any permutation of two acids is six. The theoretical combinations and actual number of glycerides obtainable for more complex acid mixtures are given in table 3.

Little more than an approach has yet been made towards the ultimate

goal of analyzing natural glyceride mixtures for the number of specific configurations indicated in table 3. Certain procedures have allowed the estimation, within limits, of the proportions of groups of component glycerides, e.g., trisaturated, disaturated-monounsaturated, monosaturated-diunsaturated, and triunsaturated, without regard to specific structures. The techniques which have proven most fruitful have aimed at separating the general structural types present in a fat into either pure fractions or essentially simpler mixtures containing a predominant amount of a given type.

Distillation in high vacuum was employed very early for the purpose of separating partially the glycerides of laurel kernel fat and nutmeg butter (189). Krafft (188, 190-192) and others (29, 30, 32, 45) used this procedure extensively to obtain glycerides of low molecular weight, e.g., trilaurin, trimyristin, lauromyristins, etc. The inability to distill glycerides of higher molecular weight limited the use of this procedure until recently, when interest was aroused in molecular distillations (44, 62, 63, 77, 95, 180, 227, 272). While the latter technique enables a distillation of high molecular weight materials without decomposition, it has not proved entirely satisfactory for the fractionation of glycerides (95, 227, 229).

Fractional crystallization offers another possible means of separating the glycerides into simpler mixtures. The early workers who chose this means had little success other than to demonstrate clearly for the first time the general occurrence of mixed rather than simple triglycerides. Amberger, Bömer, Klimont, and others (2-5, 28-35, 184-186) pursued the method assiduously for many years. In many instances they were able to effect sufficient separation to obtain qualitative identification of specific mixed triglycerides. Nothing approximating quantitative treatment resulted, however. The same is true of the fractional crystallization of brominated unsaturated fats such as linseed oil (70-72), soybean oil (93, 250), and a series of marine oils (251). Brominated mixed glycerides of oleic, linoleic, linolenic, and C_{20-22} unsaturated acids were isolated. No reference substances of the bromo derivatives reported have yet been synthesized to confirm the proposed structures and, in view of the uncertain solubilities of such derivatives (22), it would be unwarranted to expect such information to express quantitative relations. Daubert and King (59) have made an extensive study of the compounds reported in many of these investigations to determine the correlation of the isolated products with known reference structures.

The first significant quantitative method in the study of glyceride structures was developed by Hilditch and Lea (120) to measure the trisaturated glycerides. The method was based on Armstrong and Hilditch's preparation (11) of saturated esters from a mixture with unsaturated esters by

oxidation of the latter with potassium permanganate in acetone and subsequent removal of the acidic scission products from the unchanged saturated esters (*cf.* also 23). With suitable precautions, the glycerides composed entirely of saturated acids may be determined. This determination, coupled with studies of fatty acid composition, provides a means for obtaining a first approximation of general glyceride structure. When the molar proportions of the saturated acids found in the fully saturated glycerides are simply subtracted from the proportions found for the whole

TABLE 4

Proportions of general glyceride structures in various animal and vegetable fats (in molar percentages)

SOURCE OF FAT	TRISATU- RATED GLY- CERIDES	DISATURATED- MONOUN- SATURATED GLYCERIDES	MONOSATU- RATED-DIUN- SATURATED GLYCERIDES	TRIGNSATU- RATED GLYCERIDES
<i>Animal fats:</i>				
Beef tallow (14).....	25.8	33.7-53.9	40.5-0	0-20.3
Mutton tallow (58).....	26	30-52	44-0	0-22
Goat (male) (67).....	29.2	30.9-50.9	39.9-0	0-19.9
Goat (female) (64).....	30.8	29.2-49.2	40.0-0	0-14.9
Butter (147).....	33.7	36.5-51.4	29.8-0	0-14.9
<i>Vegetable fats:</i>				
<i>Pericarp fats:</i>				
Palm oil (17, 118).....	3.5-7	16.5-29	66-58	0-14
Sterculia (148).....	1	14	85	0
Piqui-a (143).....	2	42	56	0
Stillingia tallow (140).....	27.6	61-65	11.4-0	0-6
<i>Seed fats:</i>				
Coconut (55).....	84	12	4	
Palm kernel (55).....	63	26	11	
Cocoa butter (121).....	2.5	77	16	4
Borneo tallow (139).....	4.5	85	6.5	4
Shea butter (145).....	2.3	30.5-65	69.5-0	0-35
Allanblackia (145).....	1.5	63.5-82	36.5-0	0-18
Taban Merah (148).....	2	77	21	0
Cottonseed (113).....		15	60	24

original fat, the composition of the mixed saturated-unsaturated glycerides results. The latter must be mono-, di-, or tri-unsaturated. Limiting values for each group may be readily calculated from the molar proportions of saturated and unsaturated acids in the saturated-unsaturated glycerides (table 4).

An interesting relationship between the percentage of saturated fatty acids and the trisaturated glycerides has been observed as a characteristic feature of many fats. In seed fats, trisaturated glycerides do not usually

appear until the proportion of saturated acids reaches *ca.* 60 per cent. When saturated acids are present in approximately these amounts, it has been further observed that the ratio of saturated to unsaturated acids (called the "association ratio") in the non-fully saturated glycerides is 1.2 to 1.4. The proportion of saturated acids indicated by this ratio (*ca.* 60 per cent) is not exceeded even when the saturated acids in the whole fat are unusually high as, e.g., in coconut oil (94 per cent). Such findings, obtained repeatedly, indicate the general pattern of glyceride structures in the seed fats. Hilditch (100) uses these facts in support of what has come to be called a "rule of even distribution" of fatty acids throughout the glycerides of seed fats. Animal fats and some plant fats are considered by Hilditch as exceptions to this generalization, because they have a much greater amount of trisaturated glycerides for a given ratio of saturated to unsaturated acids in the whole fat. Recent studies (198), based on the most probable random distribution of any given fatty acids as glycerides (221), have indicated, however, that the animal fats are really more "evenly distributed" than are seed fats.

A further operation which has proved useful in studying glyceride structures (98, 102, 104, 110, 113, 149, 153) is the hydrogenation of fat samples, either partially or completely, with the subsequent determination of the amount and composition of the trisaturated glycerides in each hydrogenated product. Hydrogenation raises the trisaturated glycerides as the proportion of saturated acids is increased, as would be anticipated from the above remarks. The "agitation" hydrogenation process, which provides continual intimate contact of catalyst and reactants, is stepwise according to Hilditch *et al.* (41, 76, 110, 112). The disaturated-mono-unsaturated glycerides are completely saturated before any monosaturated-diunsaturated or triunsaturated molecules are fully hydrogenated. Different results are obtained by the "drip" process, in which a stationary catalyst tends to hydrogenate completely any molecules it contacts (110, 142). In completely hydrogenated vegetable oils, tristearin, palmitodistearin, and dipalmitostearin are the least soluble glycerides usually found. These may be fractionally crystallized and the approximate content of tristearin calculated from either the equivalent weights of the crystalline fractions or the setting points of mixtures of palmitic and stearic acids in the fractions. When such determinations have been made (e.g., 128) the amount of tri-C₁₈ glycerides (which are given by the tristearin figures) was close to the minimum calculated for the limiting values derived from data on component acids.

Recently a series of publications from Hilditch's laboratory (12, 42, 43, 84, 109, 127, 128, 130, 131, 133, 136-38, 151, 219) has indicated that a combination of several of the above procedures provides more informa-

tion regarding the component glycerides than had been available previously. Fractional crystallization of the whole fat in acetone solution is employed first to separate the fat into simpler mixtures of glycerides. Trisaturated glycerides are then determined on the whole fat and, in some cases, on the individual portions which have been crystallized. Each

TABLE 5

Glycerides of vegetable and animal fats

P = palmitic; S = stearic; U = unsaturated acids (oleic + linoleic)

FAT	TRISATURATED GLYCERIDES				DISATURATED- MONOUNSATURATED GLYCERIDES			MONOSAT- URATED-DIUN- SATURATED GLYCERIDES		TRISAT- URATED GLYCERIDES
	P ₁	P ₂ S	PS ₂	S ₂	P ₂ U	S ₂ U	PSU	PU ₂	SU ₂	
Mowrah (109).....		1.2			0.9		26.9	41.3	29.7	
Phulwara butter (42)..	7.9				62.4		7.2	22.5		
Shea butter (84).....		3.0	1.5			34.4		11.3	45.3	4.5
Borneo tallow (42)....	1.4	1.9	1.3	0.7	7.6	39.8	30.7	3.3	13.3	
Kokum butter (266)...				1.5	1.6	57.9	14.8	1.8	21.4	1.0
<i>Hodgsonia capniocar-</i> <i>par</i> seed fat (130)...	2.1	0.6			33.1		27.3	24.1		12.8
Margosa oil (131).....		←0.6→			5.0		12.3	26.0	33.6	22.5
<i>Allanblackia floribunda</i> kernel fat (219).....			1.3			76.0	5.4	Trace	15.4	1.9
<i>Allanblackia parviflora</i> kernel fat (219).....			1.3			60.0	9.3	Trace	26.1	3.3
Baku kernel fat (12)...			1.3			26.3	7.5	6.0	46.7	12.2
Palm oil (Cameroons) (127).....	5.1	3.3			42.7		10.8	31.5		6.6
Palm oil (Bassa) (127)...	3.0	2.7			30.5		9.9	41.5		12.4
Sapota seed oil (264)...							7.8	59.4*	27.8	5.0
Ox depot fat (136)....	3.4	7.8	5.8	0.4	14.7	2.3	32.0	22.7	10.9	Trace
Pig, outer back fat (138).....	0.6	2.4	2.4		5.4		34.0	45.4	←9.8→	
Pig, perinephric fat (138).....	0.1	4.3	4.7		9.0		39.2	35.2	←7.5→	
Cow depot fat (133)...	3.0	22.6	10.0		18.2		35.4	9.7	←0.8→	
Cow depot fat (133)...		16.5	11.8		11.0	1.8	41.6	14.2	←3.1→	

* Included in this figure is 22.8 per cent myristodiolin.

portion is further analyzed for (a) quantitative distribution of fatty acids by ester fractionation and (b) content of tri-C₁₈ glycerides by measuring the tristearin present after complete hydrogenation. From these data and the general order of solubility of known individual glycerides, it is possible to deduce the approximate glyceride composition of the original fat. The results of some of these determinations are assembled in table 5.

In addition to the determinations shown in table 5, other contributions have appeared from Hilditch's laboratory which cite the successful use of this method for solid fats (12, 43, 88, 137).

One of the limitations in these studies has been the inability to distinguish between various general types of tri- C_{18} glycerides, such as steardoileins, stearoöleolinoleins, stearolinoleins, oleolinoleins, triolein, and trilinolein, all of which are ultimately determined in the form of tristearin after complete hydrogenation. This fact makes the figures for steardo-diunsaturated and triunsaturated glycerides in table 5 less certain than, for instance, the values for the trisaturated glycerides. The greatest difficulties arise in the technologically important liquid fats (both "drying" and "non-drying" oils) in which the component acids are predominantly C_{18} and unsaturated, and, consequently, the content of trisaturated glycerides is negligible.

In order to differentiate the glyceride structures in the more unsaturated liquid fats, the latter have been converted to more solid fats by geometrical isomerization (elaidinization), using the oxides of nitrogen or sulfur (85, 113, 105, 106) or selenium (24, 88, 154). Mixed oleoglycerides are transformed by this process into mixed elaidoglycerides. The latter were studied directly in the earlier work and were resolved, more recently, into fractions of different solubility in acetone. Determination of the saturated, elaidic, oleic, and linoleic acids affords data from which the proportions of the mixed glycerides present may be approximately deduced for the elaidinized fat. The order of accuracy of quantitative conclusions drawn from results obtained in this manner is definitely lower than that which may be obtained for (solid) fats with an originally higher content of saturated acids. The polymerization of linoleic acid has been considered in these studies, but not its geometrical isomerization (173, 214).

Recently, two promising methods for treating the mixed saturated-unsaturated glycerides have been proposed. The device of low-temperature crystallization, used so effectively by Brown and his associates for the purification of unsaturated acids (36-40, 238, 239), gave Hilditch and Maddison (128) a sufficient separation of cottonseed oil to enable them to draw quantitative conclusions, within limits, as to the types of glycerides present, from considerations of the component acid analyses and the ratio of oleic to linoleic acid in each fraction. Vidyarthi and Mallya (265) have also contributed to the component glyceride analysis of similar liquid fats by a systematic examination of (debrominated) products obtained from fractions of brominated glycerides separated from suitable solvents,—a procedure which was also suggested, but not reported upon in detail, by Gunde and Hilditch (88). A limitation of the latter method which has

not received due consideration is the known incomplete and variable separation of unsaturated components as bromine addition products (22, 37, 82, 111, 214, 230, 232).

In the continuation of attempts to find methods for the preliminary resolution of complex glyceride mixtures two techniques, important commercially but not yet used in connection with problems of glyceride structure, seem promising on the basis of preliminary results. Solvent extraction of such oils as linseed, soybean, fish, perilla, and cottonseed for the selective removal of glycerides of different degrees or types of unsaturation is described by Freeman (78). Certain types of non-reactive, organic, polar solvents, notably those which are not completely miscible with the glycerides at low temperatures, are used preferentially to dissolve the unsaturated glycerides. Selective chromatographic adsorption (273) appears to provide somewhat similar separations of both fatty acids and glyceride mixtures (and also sterols and phospholipids) (47, 175, 179, 213, 256, 257). Especially in consideration of Kaufmann's work (175) does this procedure seem valuable for separations of glycerides. There are not yet sufficient data available describing either the relative adsorbability of adsorbents or the action of various solvents.

Little can be said with assurance of the specific configurations present in natural fats. The determination of configuration patterns is the next great task. There have been several instances (17, 79, 94, 112, 113, 128, 134, 145, 148, 151, 185) of the isolation of derivatives of glycerides from several natural fats in which the β -position of the glycerol molecule was occupied by the acid present in lesser quantities. However, the evidence is too scanty for more than a tentative working conception that this finding may appear generally. Such selective production of one isomeric form of a triglyceride may mean the operation of specific biological process.

It seems important to indicate that the determination of glyceride configurations is preferably based upon physical rather than chemical properties. For this reason, the extensive studies on the synthesis of glycerides of known configuration (59) and the studies of their physical properties,—such as melting and transition points and crystal structures (46, 53, 210–212), spectrographic absorption characteristics (69, 220), heats of combustion and specific heats (51, 52), dielectric properties (26, 194), and activation energies (267),—are the more valuable.

Such, in brief, is the present state of our knowledge of the structural relations of natural fats. That considerable progress has been made during the past quarter of a century is self-evident. In view of the strategic significance of certain types of natural fats, it is not unreasonable to anticipate a quickened pace in similar investigations. The broad outlines have

been defined in detail in many instances. It is, however, entirely possible that modifications of the existing conceptions may be necessary, as a result of further research.

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STRUCTURAL PECULIARITIES OF ACID-FAST BACTERIAL LIPIDS¹

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I. INTRODUCTION

The lipids contained in certain acid-fast bacteria have been under investigation in this laboratory during the past several years. This work has been carried on in coöperation with the Medical Research Committee of the National Tuberculosis Association, with the object of determining first of all the nature of biologically active compounds and their relation to the pathology of tuberculosis in man and animals. Incidentally, it has been our privilege to study the chemical composition of these compounds and to determine the various cleavage products which are liberated on saponification or hydrolysis of the several complex fractions that have been encountered.

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The lipids elaborated by higher plants and animals are relatively simple in chemical structure. For instance, the fats or glycerides are esters of fatty acids with glycerol. The phosphatides are more complex and are represented by lecithin, cephalin, and sphingomyelin. The waxes occurring in plants and animals vary in composition, but by definition they are esters of higher alcohols with higher fatty acids. The nature of the alcohols and the acids may vary, and often the waxes contain mixtures of several such component esters.

The lipids occurring in acid-fast bacteria are built on quite different plans from those found in plants and animals and their composition is more complex. Up to the present time we have examined the lipids contained in the human tubercle bacillus (1), the bovine (18) and avian types (17) of tubercle bacilli, the so-called leprosy bacillus (41), and the non-pathogenic timothy grass bacillus (26). The different strains of bacilli were grown under identical conditions on the Long synthetic medium (31), which has the following composition: asparagine, 5 g.; ammonium citrate, 5 g.; monopotassium phosphate, 3 g.; sodium carbonate, 3 g.; sodium chloride, 2 g.; magnesium sulfate, 1 g.; iron ammonium citrate, 0.05 g.; glycerol, 50 g.; water, 1000 cc.

The cultivation of the bacteria under uniform conditions on the same synthetic medium was regarded as of prime importance in the comparative studies on the composition of the acid-fast bacteria included in these investigations. The different chemical compounds contained in the bacteria would therefore reflect differences in metabolic activity, depending upon inherent variation in the life processes of the bacterial cells. The results of our investigations have demonstrated that every strain produces lipids peculiar to itself and that no two are alike, although in many respects their properties are similar.

It must be admitted that none of these studies is complete, but we believe that a general outline is evident and that the results that we have obtained can be reproduced, but certain reservations are necessary. It has been found (27), for instance, that five different strains of the human tubercle bacillus which were cultivated under identical conditions showed great variation in chemical constants and in the proportions of the various compounds. However, they all contained the same characteristic components although in varying amounts. It is probable, therefore, that every batch of bacteria that is grown will show some variation in the amount and kind of chemical compounds that are elaborated.

II. THE ACETONE-SOLUBLE FATS OF ACID-FAST BACTERIA

The acetone-soluble fats of acid-fast bacteria are mixtures of neutral fat and large amounts of free fatty acids. In addition to ordinary fatty acids

such as palmitic, stearic, oleic, linoleic, etc., the bacterial fats contain certain new and specific fatty acids. The fat of the human tubercle bacillus differs in its component fatty acids from other members of the acid-fast group, in that it contains a series of new and previously unknown acids. Two of these acids,—namely, tuberculostearic acid (8) and phthioic acid (7),—have been obtained in pure form. Tuberculostearic acid (37) has the formula $C_{19}H_{38}O_2$ and its constitution is expressed as 10-methylstearic acid, $CH_3(CH_2)_7CH(CH_3)(CH_2)_8COOH$. It is optically inactive. It melts at 10–11°C. and is an oil at ordinary temperature. Phthioic acid corresponds in composition to a saturated hexacosanoic acid, $C_{26}H_{52}O_2$. It is a thick oil at ordinary temperature and melts at about 20°C. It is dextrorotatory; $[\alpha]_D^{20} = +12.6^\circ$. The chemical constitution of phthioic acid has not yet been established, but the acid undoubtedly contains a branched chain (38). Other levorotatory acids of unusual constitution are present in the fat, but they have not been obtained in pure form (7).

The fat of the human tubercle bacillus does not contain glycerides. The water-soluble component of this fat was finally identified as the crystalline disaccharide trehalose (13). In analyzing the fats of the avian and bovine tubercle bacilli (23) and of the timothy grass bacillus (34), glycerol could not be found nor could the water-soluble compounds be identified. When these analyses were made, we were under the impression that the fats were glycerides, and attempts were made to purify the water-soluble constituents by distillation *in vacuo*. During the distillation the materials decomposed and carbonized. In no case did any glycerol distill over. Whether these fats contain trehalose will remain a problem for future investigation. However, the fat of the so-called leprosy bacillus does contain trehalose (14).

While the presence of trehalose has been demonstrated by actual isolation in crystalline form only in the fats of the human tubercle bacillus and in the leprosy bacillus, it is not impossible that the same carbohydrate functions as the alcoholic component in the fats of the other acid-fast bacilli. It is a curious fact that organisms growing on a medium in which glycerol is the chief source of carbon synthesize a complex compound such as the disaccharide trehalose, with which fatty acids are combined to form the neutral fat.

III. THE PHOSPHATIDES OF ACID-FAST BACTERIA

The phosphatides prepared from acid-fast bacteria resemble ordinary phosphatides in solubility (2, 19, 20, 22, 32). They are easily soluble in ether, and they are precipitated from ether solution with acetone. They form colloidal solutions in water. In other respects, however, the bacterial phosphatides differ entirely from the usual phosphatides of plant or animal

origin. The bacterial phosphatides contain from 2.6 to 3.5 per cent of phosphorus and very small amounts of nitrogen. The phosphorus is present in organic combination, partly as glycerophosphoric acid and partly as a phosphorylated polysaccharide or glycoside (12). The nature of the nitrogen compound has not been established, but a part of the nitrogen is present as ammonia. In no case could either choline or aminoethyl alcohol be found.

All of the bacterial phosphatides that we have examined yield on hydrolysis from 33 to 40 per cent of water-soluble compounds. The water-soluble compounds on complete hydrolysis with dilute sulfuric acid yield a small amount of inorganic phosphoric acid and a larger quantity of organic phosphoric acids which are similar in composition to glycerophosphoric acid. In addition to the phosphoric acids mentioned above, the hydrolyzed solutions contain mannose (16), inositol (6), and a hexose which on treatment with phenylhydrazine gives typical glucosazone (21). The water-soluble hydrolysis products of the bacterial phosphatides are therefore very similar.

In the examination of the component fatty acids we found that every phosphatide differed. The saturated fatty acids were represented chiefly by palmitic acid and the unsaturated acid was chiefly oleic acid, because on catalytic reduction stearic acid was always obtained. However, an unsaturated C_{18} acid was present in some cases, particularly in the acids from the phosphatide of the leprosy bacillus. In addition to these common fatty acids, every phosphatide contained representatives of saturated branched-chain acids of higher molecular weight which were oils at room temperature. The liquid saturated acids obtained from the human tubercle bacillus were dextrorotatory, while the liquid saturated acids from the other acid-fast bacteria were optically inactive.

IV. THE WAXES OF ACID-FAST BACTERIA

The so-called waxes of the acid-fast bacteria are quantitatively the most important of the ether-soluble constituents of these organisms, and from a chemical point of view they are exceedingly interesting compounds. The term "wax" for these substances is perhaps a misnomer, since they are mainly esters of optically active hydroxy acids with carbohydrates; nevertheless we have retained this term because of previous usage.

The waxes discussed in this section were obtained by extraction with chloroform after the bacteria had been exhaustively extracted with a mixture of alcohol and ether. They were purified by precipitation from ether or chloroform solution by addition of acetone or methyl alcohol until white amorphous powders were obtained. All the waxes that we have analyzed differed in composition with each strain of bacilli and were apparently

mixtures consisting of the above-mentioned esters of hydroxy acids with carbohydrates together with small amounts of true waxes; sometimes glycerides were also present. The presence of true waxes was indicated by the fact that all the waxes so far examined contained higher secondary alcohols. The waxes of the avian tubercle bacillus, the timothy bacillus, and the leprosy bacillus contained the two alcohols *d*-2-eicosanol and *d*-2-octadecanol. The wax of the human and bovine tubercle bacilli contained a different alcohol,—phthiocerol.

The polysaccharide components of the waxes are unusually interesting. The wax of the human tubercle bacillus contains a specific polysaccharide which has not been found in the waxes of the other acid-fast bacteria. On the other hand, the waxes of the avian tubercle bacillus and the timothy bacillus contain the disaccharide trehalose, while the only water-soluble component of the leprosy bacillus wax was glycerol.

A. WAXES FROM THE HUMAN TUBERCLE BACILLUS

Three wax fractions obtained from the human tubercle bacillus have been analyzed. The crude wax obtained by chloroform extraction, as mentioned above, gave on purification two fractions,—(1) the purified wax (3) and (2) the soft wax (4); fraction 3 was obtained from the mother liquors in the purification of the phosphatide (42). The composition of these fractions is described below.

1. *The purified wax*

This fraction was a white amorphous powder which melted with decomposition at 200–205°C. It contained 0.41 per cent of phosphorus and 0.77 per cent of nitrogen. The substance is extremely stable toward acids and may be refluxed with aqueous acid or heated in an autoclave at 120°C. for several hours without undergoing any noticeable hydrolysis. However, when it is refluxed with acidified alcohol it is gradually hydrolyzed, with liberation of ether-soluble compounds and water-soluble carbohydrate. While the wax is very resistant to hydrolysis by acid, it is very sensitive to alkali. If the wax is dissolved in benzene and an alcoholic solution of potassium hydroxide is added to the solution at room temperature, a gelatinous precipitate separates almost immediately. The precipitate consists of a complex water-soluble polysaccharide, whereas the ether-soluble constituents remain in the benzene solution.

The polysaccharide of the purified wax. The polysaccharide (36) on purification is obtained as a white amorphous powder which is somewhat hygroscopic. The substance contains phosphorus and nitrogen, and it gives a precipitin reaction with immune horse serum in dilutions up to 1:1,000,000. On hydrolysis with dilute acids, the polysaccharide yields

reducing sugars amounting to about 57 per cent, calculated as glucose. The reducing sugars were separated as hydrazones and identified as mannose, *d*-arabinose, and galactose, while a trace of glucosamine was isolated as the hydrochloride. A trace of inositol was also found, but the bulk of the non-reducing portion of the polysaccharide molecule could not be identified.

The amounts of reducing sugars obtained from the polysaccharide are shown in table 1.

The occurrence of *d*-arabinose in the polysaccharide is a matter of interest, because this pentose is very seldom found in nature, and is an indication of the peculiar metabolic activity of the tubercle bacillus. Heidelberger and Menzel (29) isolated *d*-arabinose among the products of hydrolysis of the specific polysaccharide from the tubercle bacillus and in our work we have found the same sugar, not only in the wax polysaccharides but in the polysaccharide associated with the firmly bound lipids. It is apparent, therefore, that *d*-arabinose is the principal reducing sugar obtained on hydrolysis of all the polysaccharide fractions of the tubercle bacillus that give precipitin reactions with immune serum.

TABLE 1
Reducing sugars from wax polysaccharide

	per cent
Mannose.....	2.1
<i>d</i> -Arabinose.....	35.9
Galactose.....	17.5

The ether-soluble constituents of the purified wax. The ether-soluble compounds obtained after saponification of the purified wax consisted principally of mycolic acid (40), a small amount of lower fatty acids, and the alcohol phthiocerol (39). These components are very firmly combined and are liberated only on prolonged saponification.

Mycolic acid. The principal fatty acid contained in the purified wax was named mycolic acid; it amounted to about 56 per cent of the wax. Mycolic acid has a very high molecular weight; its probable formula is $C_{88}H_{176}O_4$. It contains one hydroxyl group and one methoxyl group to one carboxyl group. Mycolic acid melts at 54–56°C. and has a low dextro-rotation, $[\alpha]_D = +1.8^\circ$. It is a saturated compound, since it absorbs no iodine. However, when the acid is treated in chloroform solution with an excess of bromine, varying amounts of bromine are absorbed, apparently by substitution, since hydrobromic acid is liberated. Mycolic acid is acid-fast. It is the only substance that we have found in the tubercle bacillus that possesses the property of acid-fastness, and it is probably the compound which is responsible for the acid-fastness of the bacilli.

When mycolic acid is heated under reduced pressure to a temperature above $280^{\circ}\text{C}.$, it decomposes with the liberation of normal hexacosanoic acid, $\text{C}_{26}\text{H}_{52}\text{O}_2$, which distills off, leaving a practically colorless, non-volatile, unsaturated residue (5).

When mycolic acid is demethylated by heating with hydriodic acid, and the resulting monoiodonormycolic acid is reduced, hydroxynormycolic acid is formed (30). This new acid on pyrolysis is split in the same manner as mycolic acid. On treatment with more concentrated hydriodic acid, it is possible to replace both the methoxyl and hydroxyl groups with iodine. The diiodonormycolic acid thus formed gives normycolic acid, $\text{C}_{87}\text{H}_{174}\text{O}_2$, on reduction. When normycolic acid is subjected to pyrolysis, it is decarboxylated and no hexacosanoic acid is obtained. It is evident, therefore, that the liberation of *n*-hexacosanoic acid on pyrolysis depends upon the presence of the hydroxyl group in mycolic acid.

2. *The soft wax*

The fraction designated soft wax (4) was obtained on the evaporation of the mother liquors from the purified wax. After saponification, glycerol was the only water-soluble compound that could be found. The ether-soluble constituents consisted of mycolic acid, certain lower fatty acids, and the alcohol phthiocerol. Judging by these cleavage products the substance was a mixture of glycerides and some true waxes.

3. *The wax contained in the alcohol-ether extract*

The lipids which are extracted from the tubercle bacillus with alcohol-ether contain, in addition to acetone-soluble fat and phosphatide, a small amount of wax-like material (42). The latter represents a mixture of glycerides, esters of fatty acids with a carbohydrate, and true waxes. Two water-soluble compounds were obtained on saponification,—namely, glycerol and a carbohydrate. The carbohydrate on hydrolysis with dilute acid yielded only mannose and inositol, and thus has a very different composition from the specific polysaccharide contained in the “purified wax.”

The fatty acids consisted mainly of mycolic acid, but notable quantities of other fatty acids were present,—namely, *n*-hexacosanoic acid, tuberculo-stearic acid, phthioic acid, and a levorotatory acid corresponding to the formula $\text{C}_{31}\text{H}_{62}\text{O}_2$. An appreciable quantity of the alcohol phthiocerol was also present.

Phthiocerol. The alcohol phthiocerol was first isolated from the purified wax of the human tubercle bacillus by Stodola and Anderson (39). It has since been found as a constituent of all the wax fractions of the human tubercle bacillus. It also occurs in the wax of the bovine tubercle bacillus,

but it has not been found in any of the other acid-fast bacteria. Phthiocerol crystallizes from ethyl acetate in rosettes of prismatic needles, m.p. 73–74°C., $[\alpha]_D$ in chloroform = -4.8° . Its composition corresponds to the formula $C_{35}H_{72}O_3$, or possibly $C_{36}H_{74}O_3$. It contains two hydroxyl groups and one methoxyl group. The chemical constitution of phthiocerol has not yet been established.

B. THE WAX OF THE BOVINE TUBERCLE BACILLUS

The crude chloroform-soluble wax of the bovine tubercle bacillus, which was isolated by Anderson and Roberts (18), amounted to 8.5 per cent of the dried bacteria. The wax was purified and analyzed by Cason and Anderson (25). The purified wax was a white amorphous powder, having the following properties: m.p. (unsharp), 47–54°C.; $[\alpha]_D$ in benzene, $+15.5^\circ$; iodine number, 3.2. It contained 0.30 per cent of phosphorus and a trace of nitrogen. In properties and in chemical composition the wax showed an interesting similarity to the wax fraction described in the preceding paragraph, which had been obtained from the alcohol-ether extract of the human tubercle bacillus (42).

The purified wax yielded the following cleavage products on saponification: glycerol, 1.3 per cent; carbohydrate, 9.0 per cent; crude bovine mycolic acid, 61.0 per cent; lower fatty acids, 19.4 per cent; and unsaponifiable matter, 5.3 per cent.

The carbohydrate was an amorphous white powder which contained 2.2 per cent of phosphorus and a trace of nitrogen. On hydrolysis it gave mannose, inositol, and inositolmonophosphoric acid.

The purified bovine mycolic acid was very similar in properties to the mycolic acid obtained from the wax fractions of the human tubercle bacillus. It melted at 56–58°C.; $[\alpha]_D$ in chloroform = $+2.70$; molecular weight by titration, 1219. It contained one hydroxyl group and one methoxyl group to one carboxyl group. The bovine mycolic acid, when subjected to pyrolysis at a temperature of 250–300°C., decomposed in the same manner as mycolic acid. The volatile acid that distilled off was identified as *n*-hexacosanoic acid ($C_{26}H_{52}O_2$; m.p., 87–88°C.; molecular weight by titration, 399).

The examination of the lower fatty acids revealed some new interesting acids. The mixed acids were first separated by means of the lead salt-ether procedure into solid and liquid acids. The solid acids were converted into methyl esters, and the esters were fractionated by distillation *in vacuo*. The first ester fraction proved to be methyl palmitate, m.p. 27–28°C., which on saponification gave pure palmitic acid (m.p., 62–63°C.; molecular weight by titration, 252). The next ester fractions were apparently mixtures, but the highest boiling fraction gave on saponifica-

tion an acid which, after repeated recrystallization from acetone, had a constant melting point of 76–77°C. The composition (C, 78.20 per cent; H, 13.12 per cent) and molecular weight (368) are in agreement with the calculated values of a tetracosanoic acid, $C_{24}H_{48}O_2$. However, the acid differed in crystal form from the normal higher fatty acids. It crystallized from acetone in bulky diamond-shaped crystals and from methyl alcohol in boat-shaped needles. The low melting point and unusual crystal form would indicate that the acid possessed a branched-chain structure.

The acids obtained from the ether-soluble lead salts formed a semi-solid mass and had an iodine number of 5. The mixture was subjected to catalytic reduction, after which the lead salt-ether procedure was repeated. A small amount of solid reduced acid was obtained from the ether-insoluble lead salt, but the substance was a mixture from which no pure acid could be isolated. The ether-soluble lead salts yielded a mixture of acids that was converted into methyl esters. The esters on fractionation *in vacuo* gave two principal fractions.

Fraction I was an optically inactive colorless oil, b.p. 112–114°C. at 0.006 mm. After saponification the free acid was obtained as a colorless crystalline mass, m.p. 29–30°C. The analysis gave: C, 75.93 per cent; H, 12.73 per cent; molecular weight by titration, 284.5. These values agree with the calculated composition of stearic acid. The 2,4,6-tribromoanilide was prepared and recrystallized from methyl alcohol, yielding snow-white needles that melted at 96–96.5°C. This new acid is apparently a branched-chain isomer of stearic acid and an analog of tuberculostearic acid.

Fraction II distilled at 172–175°C. at 0.003 mm. and melted at 22–24°C.; $[\alpha]_D = -5.3^\circ$. After the ester had been saponified, the free acid was obtained as a colorless wax-like solid, having the following properties: m.p., 33–34°C.; $[\alpha]_D = -3.98^\circ$; molecular weight by titration, 430. This levorotatory acid was apparently a mixture, and no pure tribromoanilide could be obtained from it.

The unsaponifiable matter of the bovine bacillus wax consisted mainly of a crystalline higher alcohol (24) which separated from ethyl acetate in aggregates of colorless prismatic crystals, m.p. 73–74°C., $[\alpha]_D$ in chloroform = -4.06° . It contained two hydroxyl groups and one methoxyl group, and its composition corresponded to the formula $C_{35}H_{72}O_3$ or $C_{36}H_{74}O_3$. The above alcohol was identical in composition, crystal form, and properties with the alcohol phthiocerol, which had previously been isolated from the human tubercle bacillus wax. It is an interesting fact, and one that appears to have some significance, that the waxes of the human and bovine tubercle bacilli contain the alcohol phthiocerol, because this alcohol has not been found in the other acid-fast bacteria. Moreover, the

bovine mycolic acid resembles mycolic acid from the human tubercle bacillus and both yield *n*-hexacosanoic acid on pyrolysis. These similarities in chemical constituents of the waxes of the human and bovine tubercle bacilli indicate such a close relation between the two organisms that it sets them apart from other members of the acid-fast groups of bacteria.

The cleavage products of the bovine bacillus wax indicate that the substance was a mixture containing glycerides, fatty acid esters of a special phosphorus-containing carbohydrate, and fatty acid esters of the alcohol phthiocerol. The fatty acids were a complex mixture in which only one ordinary acid,—namely, palmitic acid,—was present. All of the other acids were new and specific compounds. The bovine mycolic acid resembled the mycolic acid of the human tubercle bacillus in properties, but its molecular weight was lower and undoubtedly the two acids differ in chemical constitution, although both gave *n*-hexacosanoic acid on pyrolysis. The wax contained also at least three new fatty acids of lower molecular weight and of unknown constitution: (a) a solid tetracosanoic acid, $C_{24}H_{48}O_2$, m.p. 76–77°C., which probably possessed a branched-chain structure; (b) a new branched-chain acid, $C_{18}H_{36}O_2$, m.p. 29–30°C., which was optically inactive and the lead salt of which was soluble in ether, (c) a mixture of higher levorotatory acids whose lead salts were also easily soluble in ether.

C. THE TIMOTHY BACILLUS WAX

The chloroform-soluble wax was purified by precipitation from ether solution by addition of acetone until a nearly white powder was obtained. The purified wax, as reported by Pangborn and Anderson (33), had the following properties: m.p., 45°C.; ash, 1.7 per cent; phosphorus, 0.29 per cent; nitrogen, 0.41 per cent; iodine number, 20.5; saponification number, 66.9; $[\alpha]_D$ in chloroform, +15.1°. After saponification the following cleavage products were isolated: glycerol, 3.0 per cent; carbohydrate, 4.6 per cent; mixed fatty acids, 68.0 per cent; neutral material, 13.0 per cent. The nature of the phosphorus and nitrogen components could not be determined.

The glycerol was identified by preparing the crystalline tribenzoyl derivative, which melted at 75–76°C. The carbohydrate fraction could not be crystallized directly, probably owing to small quantities of impurity, but after acetylation a crystalline acetyl derivative was obtained which was identified as trehalose octaacetate. This was the first time that the disaccharide trehalose had been found as a constituent of a bacterial wax.

In the examination of the fatty acids none of the ordinary acids could be found. The acids were apparently unsaturated, optically active hydroxy acids of high molecular weight, but since they did not crystallize but separated as amorphous white powders the degree of purification attained

was uncertain. What appeared to be the purest fraction corresponded in composition to an acid having the formula $C_{70}H_{138}O_6$. It was probably a dibasic acid, because the molecular weight found by titration was 518 but the Rast method gave a value of 1000. On acetylation only one hydroxyl group could be demonstrated. No methoxyl could be found. The acid melted at 56–57°C.; $[\alpha]_D$ in chloroform = +6.1°; iodine number = 15.2.

The unsaponifiable or neutral material was found to contain two new interesting crystalline secondary alcohols, *d*-2-eicosanol and *d*-2-octadecanol, which were optically active. They were the first higher alcohols to be isolated and identified as constituents of acid-fast bacterial waxes and whose chemical composition and constitution could be established.

The composition of the alcohols indicated the formulas $C_{20}H_{42}O$ and $C_{18}H_{38}O$, and on oxidation with chromic acid the corresponding ketones

TABLE 2

Properties of the two new alcohols (and their derivatives) isolated from the timothy bacillus wax

ALCOHOLS AND DERIVATIVES	MELTING POINT	$[\alpha]_D$ IN $CHCl_3$	KETONES AND DERIVATIVES	MELTING POINT
	°C.			°C.
<i>d</i> -2-Eicosanol.....	62–63	+4.2°	2-Eicosanone.....	58–59
Acetyl derivative.....	35–37	+1.5°	Semicarbazone.....	128
Benzoyl derivative....	39–40		Oxime.....	73–74
Phenylurethan.....	78–78.5			
<i>d</i> -2-Octadecanol.....	56	+5.7°	2-Octadecanone.....	52
Phenylurethan.....	72–73	+7.9°	Semicarbazone.....	127.5

were obtained in crystalline form and in nearly quantitative yield. The ketones were identified as 2-eicosanone, $CH_3(CH_2)_{17}COCH_3$, and 2-octadecanone, $CH_3(CH_2)_{15}COCH_3$. Accordingly, the alcohols possessed the following constitutions: *d*-2-eicosanol, $CH_3(CH_2)_{17}CHOHCH_3$; *d*-2-octadecanol, $CH_3(CH_2)_{15}CHOHCH_3$.

The properties of the alcohols and ketones and their derivatives are shown in table 2.

Considering the nature of the saponification products obtained, it seems evident that the timothy bacillus wax was composed of a complex mixture of solid glycerides, fatty acid esters of the disaccharide trehalose, and esters of *d*-2-eicosanol and *d*-2-octadecanol. The only previously known components were glycerol and trehalose.

D. THE AVIAN TUBERCLE BACILLUS WAX

The chloroform-soluble wax from the avian tubercle bacillus isolated by Anderson and Roberts (17) was purified and studied by Reeves and Anderson (35). The crude wax was purified by precipitation from ether

and chloroform by addition of methyl alcohol, and two purified fractions were obtained as white amorphous powders. The properties of the two fractions and their saponification products are given in table 3.

The main differences between the two fractions were in the magnitude of the optical rotation and in the percentage of carbohydrate. Fraction I had the highest rotation and the highest per cent of carbohydrate. Glycerol was absent from both fractions.

The fatty acids consisted mainly of optically active hydroxy acids of high molecular weight, which will be mentioned more fully below. The small amount of lower fatty acids evidently represented a mixture from which no pure acid could be isolated. The ordinary straight-chain fatty acids were entirely absent.

TABLE 3

Properties of the purified wax of the avian tubercle bacillus and products formed upon its saponification

	FRACTION I	FRACTION II
Properties of the wax:		
Melting point, °C.....	54-55	53-55
$[\alpha]_D$ in chloroform.....	+38.6°	+17.7°
Iodine number.....	4.5	8.7
Products formed upon saponification of the wax:		
Carbohydrate, per cent.....	12.3	5.6
Hydroxy acids, per cent.....	81.0	84.8
Lower fatty acids, per cent.....	2.4	6.8
Unsaponifiable matter, per cent.....	9.9	9.0

1. Identification of trehalose

The carbohydrate component remained as an insoluble mass in the reaction flask after the wax had been saponified with alcoholic potassium hydroxide. It was dissolved in water, and a slight precipitate which appeared on acidification with acetic acid was removed by filtration. The addition of lead acetate gave a slight precipitate, which was removed by filtration and discarded. The carbohydrate contained in the filtrate was precipitated with basic lead acetate and ammonia. The lead salt was filtered off, washed with dilute ammonia, suspended in water and decomposed with hydrogen sulfide. After removing the lead sulfide, the filtrate was concentrated to a thick syrup, and the latter was dehydrated by grinding in a mortar under absolute alcohol until a fine white powder was produced. The substance gave no color reactions for pentose, and it did not reduce Fehling's solution until it had been boiled for some time with dilute acid. The acetyl derivative was prepared and on recrystalliza-

tion from methyl alcohol was obtained as colorless prismatic needles, m.p. 97–98°C., $[\alpha]_D$ in chloroform = +163.7°. The acetic acid liberated on saponification was 70.6 per cent. The properties identify the substance as trehalose octaacetate. Trehalose itself was isolated, after the acetyl derivative had been saponified, and was crystallized from 80 per cent alcohol. Large colorless prismatic crystals were obtained, m.p. 98°C., $[\alpha]_D$ in water = +178°. The crystals lost 9.85 per cent in weight on drying, corresponding to two molecules of water of crystallization. The properties of the sugar were in complete agreement with those of the disaccharide trehalose.

2. The unsaponifiable matter

The unsaponifiable matter consisted mainly of *d*-2-eicosanol, but a small amount of *d*-2-octadecanol was also found. The two alcohols were identical in composition and properties with the alcohols first isolated from the timothy bacillus wax, and on oxidation with chromic acid the corresponding ketones, 2-eicosanone and 2-octadecanone, were obtained and identified.

3. The hydroxy acids

The hydroxy acids did not crystallize but separated as colorless fine globular particles. Attempts to purify the acids or their acetyl derivatives were not successful, but the results indicated that a mixture of acids was present. Fractionation of the potassium salts of the mixed acids resulted in the isolation of two acids that differed in composition and properties. The potassium salts were insoluble in alcohol but easily soluble in ligroin. However, on extraction of the ligroin solution with methyl alcohol a portion of the potassium salt went into the alcoholic layer. By repeating the process several times with each fraction, it was possible to obtain two acids whose properties did not change on further purification. The salt contained in the alcoholic solution gave an acid having a molecular weight of about 500. This acid was later designated as avian α -mycolic acid (9). The ligroin solution contained the salt of an acid which has a molecular weight of about 1300 and was called avian β -mycolic acid (9). The acids had low iodine numbers but when treated with an excess of bromine they gave bromo derivatives through substitution with liberation of hydrobromic acid. Both acids were acid-fast.

The properties of the acids and of some derivatives are given in table 4.

The avian mycolic acids when subjected to pyrolysis under reduced pressure were split in the same manner as mycolic acid, yielding volatile crystalline acids and leaving nearly colorless non-volatile residues. Avian α -mycolic acid on heating to 210°C. gave a volatile crystalline acid in a

yield of 25.4 per cent. This acid crystallized in branching feathery forms and was probably a branched-chain pentacosanoic acid, $C_{25}H_{50}O_2$.

The avian β -mycolic acid when heated under the same conditions to a temperature of 280°C . gave a volatile acid in a yield of 21 per cent. This acid crystallized in thin colorless plates similar to the usual crystal form of the higher normal fatty acids and its composition corresponded to tetracosanoic acid, $C_{24}H_{48}O_2$.

TABLE 4
Properties of the avian wax acids

	AVIAN α -MYCOLIC ACID	AVIAN β -MYCOLIC ACID
Melting point, $^\circ\text{C}$	69-70	60-61
$[\alpha]_D$ in chloroform.....	+5.6°	+5.5°
Analysis, per cent.....	C, 78.99; H, 12.78	C, 82.46; H, 13.49
Molecular weight by titration...	501-520	1280-1300
Acetyl derivative, m.p., $^\circ\text{C}$	54-55	48-57
Methyl ester, m.p., $^\circ\text{C}$	54-55	49-50
Active hydrogen of ester, per cent.....	0.92	0.82
Bromo derivative, m.p., $^\circ\text{C}$	47-49	43-49
Bromo derivative, per cent bromine.....	22.4	22.9
Iodine number, Hanus.....	6.5	5.5

TABLE 5
Properties of the volatile acids obtained by pyrolysis of the avian mycolic acids

PROPERTIES	AVIAN α -MYCOLIC ACID	AVIAN β -MYCOLIC ACID
Melting point, $^\circ\text{C}$	78-79	83
Carbon, per cent.....	78.67	78.30
Hydrogen, per cent.....	13.23	13.05
Molecular weight by titration.....	380	368
Formula.....	$C_{25}H_{50}O_2$	$C_{24}H_{48}O_2$

The properties of the acids obtained on pyrolysis of the avian mycolic acids are given in table 5. The acids produced by pyrolysis were undoubtedly homogeneous, because the melting points of the top fractions were identical with those of the acids recovered from the mother liquors.

Attention is called to the fact that there is an interesting similarity in the components of the wax of the avian tubercle bacillus and of the timothy bacillus wax. From both waxes the disaccharide trehalose was obtained and they also contained the same higher alcohols,—namely,

d-2-eicosanol and *d*-2-octadecanol. The hydroxy acids also possessed similar properties but they were not identical. Neither wax contained any of the ordinary straight-chain normal fatty acids.

E. LEPROSIN

In the purification of the phosphatide of the so-called leprosy bacillus as described by Uyei and Anderson (41), a considerable amount of wax-like material was recovered from the mother liquors. This material, which was called leprosin, was purified and its composition was studied by Anderson, Crowder, Newman, and Stodola (11). Purified leprosin was a white amorphous powder, having the following properties: m.p., 50–51°C.; $[\alpha]_D$ in chloroform, +4.0°; iodine number, 5. Analysis showed that it contained only carbon, hydrogen, and oxygen.

The following products were obtained on saponification of leprosin: glycerol, 6.0 per cent; mixed fatty acids, 93.0 per cent; and unsaponifiable matter, 7.0 per cent. The only water-soluble constituent that could be found was glycerol, and it was identified by means of the tribenzoyl derivative.

The fatty acids were composed of a complex mixture in which at least six different acids were present. The ordinary normal fatty acids were represented by myristic, palmitic, stearic, and tetracosanoic acids. There was also present a notable amount of an acid which appeared to be a tricosanoic acid.

The most interesting component fatty acid was a new optically active hydroxy acid named leprosinic acid, which amounted to about 20 per cent of leprosin. The properties of leprosinic acid were similar to those of other hydroxy acids which we have found in the bacterial waxes, and it also was acid-fast. It melted at 62–63°C.; $[\alpha]_D$ in chloroform = +4.0°; iodine number = 6. The exact formula could not be established, but the simplest formula calculated from the analytical values was $C_{44}H_{88}O_3$. It is probable, however, that leprosinic acid is a dibasic acid, and the above formula should therefore be doubled.

The unsaponifiable matter consisted largely of the secondary alcohol *d*-2-eicosanol, but a small amount of *d*-2-octadecanol was also present.

It is evident from the cleavage products obtained on saponification that leprosin is a mixture of solid glycerides and esters of fatty acids with the two higher alcohols mentioned above. Leprosin is the only wax-like substance obtained from acid-fast bacteria—except the soft wax of the human tubercle bacillus—that we have examined that was free from carbohydrate. It is noteworthy that leprosin contained such a large proportion of normal fatty acids, from myristic to tetracosanoic acid. The phosphatide and the acetone-soluble fat of the leprosy bacillus like-

wise contained a large proportion of normal fatty acids,—acids which occur to but a small extent in the lipid fractions of the other acid-fast bacteria. However, the presence of the hydroxy acid, leprosinic acid, and the secondary alcohols *d*-2-eicosanol and *d*-2-octadecanol indicates a family resemblance of the leprosy bacillus to some other members of the acid-fast group of bacteria, notably to the timothy grass bacillus and to the avian tubercle bacillus.

V. THE FIRMLY BOUND LIPIDS

The so-called firmly bound lipid of the acid-fast bacteria is so strongly combined in the cellular structure that it can not be removed by extraction with neutral solvents. The bacterial residues, after exhaustive extraction at room temperature with a mixture of equal parts of alcohol and ether, followed by extraction with chloroform, contain only traces of lipid that is soluble in neutral solvents. However, after the bacterial residues have been treated with a mixture of alcohol and ether containing 1 per cent of hydrochloric acid, they will yield from 8 to 19 per cent of additional lipids on extraction with ether and chloroform. The greater portion of the bound lipids can be obtained as white amorphous powders by precipitation from ether or chloroform solutions by addition of alcohol. The mother liquors yield small quantities of soft salve-like material. The solid products are easily soluble in ether, chloroform, benzene, and ligroin, but nearly insoluble in alcohol or acetone.

So far only the solid purified fractions obtained from the human (15) and avian (10) tubercle bacilli and from the leprosy bacillus (28) have been examined in some detail. While the solid fractions of the firmly bound lipids are easily soluble in ether or chloroform, it has been found that, when such solutions are forced through a Chamberland filter, only a part of the dissolved material will pass through the filter. We have thus obtained filterable and unfilterable fractions. However, solutions of the firmly bound lipid of the leprosy bacillus passed completely through the Chamberland filter.

The results of the analyses of the firmly bound lipids are summarized in table 6. It will be noted that the unfilterable lipids contained twice as much polysaccharide as the filterable fractions.

The cleavage products obtained from the firmly bound lipids on saponification indicate that the substances were principally esters of hydroxy acids with polysaccharides. The filterable bound lipids from the human tubercle bacillus contained, however, a notable quantity of lower fatty acids, among which tuberculostearic acid was identified but from which phthioic acid was absent.

The hydroxy acid from both the unfilterable and the filterable fractions

of the bound lipids of the human tubercle bacillus appeared to be identical with mycolic acid. The alcohol phthiocerol, which occurs in the wax fractions, was entirely absent in the bound lipids. The polysaccharide was similar in composition to the polysaccharide contained in the purified wax (36).

The hydroxy acid contained in the bound lipids of the avian tubercle bacillus differed in properties from avian α - and β -mycolic acids and was called avian γ -mycolic acid. On pyrolysis it decomposed with the liberation of 18 per cent of a crystalline acid that distilled off. The volatile

TABLE 6
Percentage composition of firmly bound lipids

	UNFILTERABLE		FILTERABLE		
	Human	Avian	Human	Avian	Leprosy
Firmly bound lipid.....	4.7	2.68	7.5	8.16	19.5
Polysaccharide.....	50.5	31.3	25.5	15.0	40.5
Glycerol.....	None	None	2.0	None	None
Hydroxy acids.....	51.0	52.8	41.1	69.0	56.3
Lower fatty acids.....	4.0	3.8	28.4	3.7	4.3
Neutral material.....	None	10.6	0.8	8.2	5.5

Percentage composition of the polysaccharides

Precipitin reaction.....	1:1,000,000	1:500,000			1:2,000,000
Phosphorus, per cent.....	0.38	1.04			None
Nitrogen, per cent.....	0.95	1.81			Trace
Reducing sugar on hydrolysis, per cent.....	57.0	50.1			50.5
Mannose, per cent.....	6.6	27.3			None
<i>d</i> -Arabinose, per cent.....	38.7	19.9			41.4
Galactose, per cent.....	12.2	3.5			1.0
Inositol.....	Present	Present			None
Glucosamine.....	Present	Present			None

acid corresponded in composition to a tetracosanoic acid, $C_{24}H_{48}O_2$, which probably had a branched-chain structure. The neutral material contained the two secondary alcohols *d*-2-eicosanol and *d*-2-octadecanol.

The polysaccharide component gave a precipitin reaction with immune serum. On hydrolysis, reducing sugars were liberated and identified as mannose, *d*-arabinose, and galactose. The composition of the polysaccharide was therefore strikingly different from that of the carbohydrate contained in the wax fraction, which consisted of the disaccharide trehalose.

The hydroxy acid contained in the bound lipids of the leprosy bacillus appeared to be identical in properties with leprosinic acid (11). On pyrol-

ysis it decomposed in the same manner as the mycolic acids. The volatile acid that distilled off was apparently a new branched-chain tetracosanoic acid. The neutral material contained the same alcohols as were present in leprosin,—namely, *d*-2-eicosanol and *d*-2-octadecanol.

The polysaccharide gave a high precipitin reaction with immune serum, but it differed in composition from all the polysaccharides that we have found in the wax fractions of acid-fast bacilli. On hydrolysis, about 50 per cent of reducing sugars were liberated and 41 per cent of the polysaccharide was identified as *d*-arabinose. The presence of some other pentose was indicated, but it could not be identified. The only other reducing sugar that could be isolated was galactose. No mannose could be found.

All of the bacterial polysaccharides that we have studied,—from the purified wax of the human tubercle bacillus as well as from the above-mentioned firmly bound lipids,—give about 50 per cent of reducing sugars on hydrolysis. We have been able to account for practically all of the reducing sugars as mannose, *d*-arabinose, and galactose by actual isolation by means of appropriate hydrazones. Unfortunately it has not been possible to identify the non-reducing portion of the polysaccharides.

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THE STRUCTURE OF THE PHOSPHOLIPIDS^{1, 2}

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I. INTRODUCTION

Fourcroy (13) in 1793 was probably the first to find indication of the occurrence of complex fatty compounds. About nineteen years later Vauquelin (58) isolated from the brain fatty materials that contained phosphorus. In the next few years this type of compound was obtained by other investigators from various sources, nearly all of which were animal in nature. These preparations were in all probability very impure mixtures of substances now known as galactolipids, lecithins, cephalins, and sphingomyelins.

Strecker (49) identified the nitrogen base in these fatty compounds as the same type of nitrogen compound he had previously discovered in bile and gave it the name of choline. The rest of the structure of the molecule was furnished by Diaconow (8) and Strecker (50), when they showed that two molecules of fatty acid, either alike or different, and a molecule of glycerol could be isolated from the hydrolytic products of these fatty

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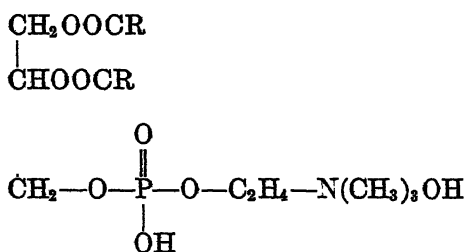
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compounds. The fatty acids were thought to be esterified with the glycerol.

Of all the different phospholipid materials that have been found in animal tissue, three compounds of particular interest and importance have been isolated and studied,—namely, lecithin, cephalin, and sphingomyelin. It is with these three that this paper will deal. The structure and properties of these compounds were thoroughly covered in a classical monograph by MacLean and MacLean (39), and since that time not a great deal of change is to be found in the understanding of their structure, with the possible exception of cephalin.

II. LECITHIN

Diaconow (8) and Strecker (50) satisfactorily showed that the lecithin molecule consisted of two fatty acid radicals esterified to glycerol and a phosphoric acid radical attached both to the third hydroxyl group of the glycerol and to the nitrogenous basic group choline. These investigators did not agree, however, as to the manner in which the choline was attached to the phosphoric acid radical. The combination could be accomplished in two ways,—either through the hydroxyl group of the choline nitrogen as a salt, or through the hydroxyl of the ethanol group of the choline as an ester. The ester form of choline union, championed by Strecker, was later confirmed by Hundeshagen (21) and Gilson (15). The structure commonly accepted agrees in general with that given by MacLean and MacLean (39) as:



where the R groups are fatty acid radicals.

In this formula the β -carbon atom of the glycerol is asymmetric, and thus the compound should be optically active. Ulpiani (57) showed that lecithin is optically active. Three years later Willstätter and Lüdecke (59) isolated from the hydrolytic products of lecithin an optically active glycerophosphosphoric acid. This optical activity would seem to indicate that the above structure for lecithin is correct, rather than that of the possible isomer in which the cholinephosphoric acid radical is attached to the β -carbon atom of glycerol. This latter structure, however, may also occur in nature. Fischgold and Chain (10) indicate the possibility

that lecithin exists as the endo salt, in which the hydroxyl of the choline nitrogen and that of the phosphoric acid group lose a molecule of water. They find that in benzene-alcohol solution lecithin combines with one equivalent of hydrogen ions in acid solution.

The products of the hydrolysis of lecithin have been shown to be choline, phosphoric acid, glycerol, and fatty acids. The complete separation of lecithin from cephalin has been difficult to carry out, but when it is successful, the lecithin contains no trace of amino nitrogen, leaving choline as the sole base present. The presence of glycerol in lecithin was proven much earlier, since the complete separation from cephalin was not necessary. Gobley (16) in 1850 proved the presence of glycerol in the lecithin from certain fish eggs, and later Foster (12) found that the amount of glycerol present conformed to the theory. As to phosphoric acid and its proportion, the results of the older investigators are acceptable. Here again, the presence of cephalin in the samples of lecithin does not appreciably change the phosphorus content.

Much has been written about the fatty acid content of lecithin. Samples of lecithin from various sources, as brain, liver, egg yolk, and soybean, have been shown to contain identical saturated fatty acids,—palmitic and stearic. Among the unsaturated fatty acids found were oleic, linolic, arachidonic, and linolenic. The amount of saturated fatty acid was usually about equal to the amount of unsaturated fatty acid found; however, Sinclair (48) has shown that the proportion of unsaturated fatty acid in animal lecithins can be greatly increased by feeding a diet high in unsaturated fatty acids.

A zwitter-ion structure has been proposed for lecithin as well as for the other phospholipids. Thus it would be expected to show varying electrophoretic velocities in solutions of varying pH. Recently, Bull and Frampton (2) determined the isoelectric points of lecithin containing various amounts of cephalin, and found the pH to be dependent upon the amount of cephalin present as determined by the amino nitrogen content. Their isoelectric point for pure lecithin upon extrapolation of experimental values was at pH 6.4. This agrees well with that found by Chain and Kemp (3), who report a pH of 6.7 ± 0.2 . A calculation of the isoelectric point from dielectric constant data places it at pH 7.5. The discrepancy here is attributed by them to decomposition of the lecithin, but Bull and Frampton question this conclusion. This spontaneous decomposition of lecithin, even when air was excluded, was reported by Price (44). Fischgold and Chain (10) found that the electrophoretic mobility increased with increased age of the lecithin suspensions. They also investigated the acid- and base-binding capacities of lecithin and other phospholipids in benzene-alcohol solution, finding that in alkaline solution

only those containing an amino group (the cephalin) can give off one equivalent of hydrogen ions. In acid solution, all the phospholipids were found to take up one equivalent of hydrogen ions. From this they assume that lecithin can exist only as a zwitter ion or as a cation. They strongly discount the existence of the "hydrate formula." Thudichum (56), Koch and Pike (28), Koch and Todd (29), and Peters and Man (43) have reported combination of ions with phospholipids. Christensen and Hastings (6) report on extensive studies of the binding capacities of phospholipids and inorganic salts. They find that fresh lecithin in aqueous solution does not bind sodium, potassium, or chloride ions. Lecithin showed no buffering over a pH range of 3 to 9 by electrometric titration.

In the several attempts to synthesize lecithin, Hundeshagen (21) obtained choline distearyl glycerophosphate by treating distearyl phosphoric acid with choline carbonate. This product was a choline salt and not the ester. Later Grün and Kade (18) obtained the choline chloride ester of distearyl phosphoric acid (lecithin chloride), which agreed with lecithin in general solubility in many organic solvents but was obtained in insufficient quantity for positive identification. Grün and Limpächer (19) synthesized a distearyllecithin and established its constitution. No natural lecithin of this composition had been isolated; however, it had been prepared by hydrogenating purified lecithin from egg yolk. Recently Kabashima (22) obtained a dipalmitoyl- β -lecithin which showed many of the solubility properties of natural lecithin. Kabashima (23) also prepared a lysolecithin that resembled the natural product but possessed a somewhat smaller hemolytic action.

III. CEPHALIN

Apparently the first clear differentiation of cephalin from lecithin was made by Thudichum (56), who separated them by the insolubility of cephalin in alcohol. He considered the essential constituents to be cephalic acid, stearic acid, glycerophosphoric acid, and choline (see MacLean's (39) conclusion that Thudichum's "neurin" was properly choline) and suggested two possible structures. One of these, supported by the tendency of part of the phosphorus to cling to the fatty acids after hydrolysis, connected the fatty acids and glycerol directly to the phosphoric acid, with the choline replacing a hydroxyl group in the glycerol. The other structure was like that now accepted for both lecithin and cephalin. Cephalic acid, a fatty acid with seventeen carbon and three oxygen atoms, was proposed as an indispensable constituent, with various cephalins possible as a result of the replacement of the stearic acid by lower saturated acids or by unsaturated acids. Aminoethanol and another base not clearly defined were found, but were considered as probable decomposition products of the choline.

Stearic acid has been amply confirmed in cephalin, and is believed by Cousin (5), Parnas (41, 42), Levene and West (34), MacArthur and Burton (37), Levene and Rolf (33), and Klenk (25) to be the only saturated acid present in substantial quantities in brain cephalin, although traces of palmitic acid were found. Fränkel and Dimitz (14) reported considerable quantities of palmitic acid, but the soundness of their conclusion is questioned by Parnas. Nishimoto is quoted by Klenk and Schuwirth (27) as reporting considerable quantities of palmitic acid in egg cephalin.

Apparently the cephalic acid reported by Thudichum (56) was a mixture of partially oxidized unsaturated acids, and in view of the preponderance of unsaturated acids, it is not strange that he considered it the most important group. Parnas (41) probably did the first extensive work on the identification of the unsaturated acids. He concluded that linolic acid was the chief unsaturated acid present, and that the small quantities of oleic acid and of an acid with three double bonds found were probably derived from impurities. MacArthur and Burton (37), however, reported that oleic acid was present as about 50 per cent of the total fatty acids, and also observed indications of acids containing three and four double bonds. Support for their contention was afforded by Levene and Rolf (33), who isolated oleic and arachidonic acids and found evidence of some other unsaturated acids which they failed to identify positively. Klenk (25) hydrogenated the unsaturated acids of brain cephalin and found only stearic and behenic acids. He questioned whether Levene and Rolf might have been mistaken in claiming arachidonic acid. Klenk's experiments indicated that the twenty-two-carbon-atom unsaturated acid had at least four, possibly five, double bonds, and he suggests that it may be the elupanodonic acid of Tsujimoto. Nishimoto's results (see Klenk and Schuwirth (27)) on egg cephalin agreed with those of Levene and Rolf on brain cephalin in that he reported oleic and arachidonic acids. Sinclair's (48) work showed that one unsaturated acid may replace not only another unsaturated acid but also a saturated acid in cephalin as well as in lecithin, and it is quite generally accepted that different samples of cephalin differ in the fatty acids present. The wording of Sinclair's conclusions encourages a misinterpretation of his meaning. His first statement is clear: "One class is composed of the more highly unsaturated phospholipids, and functions in the essential make-up of the cell; the other class, consisting of the less unsaturated phospholipids, functions as an intermediary product in the metabolism of fat." The confusion is likely to enter when he describes the more highly unsaturated compounds as the "non-metabolic type." These phospholipids, chiefly cephalins, are undoubtedly highly important in cell metabolism; Sinclair is merely pointing out that the other type apparently functions primarily in fat metabolism.

More critical is the controversy over the nitrogen base in cephalin. Thudichum (56) thought it was choline, and Cousin (5) held that view as late as 1907, but practically all other workers in the field believed aminoethanol to be the base of cephalin, and that any choline found came from lecithin as a result of the intersolubility of the phospholipids. Such reviews as those of Mathews (40), MacLean and MacLean (39), and Thierfelder and Klenk (55) reflect this view, but mention the work of MacArthur (36) in which he reported the presence of an amino acid, supported by a copper method. Mathews (40, page 592) quoted MacArthur as finding aminoöxybutyric acid and serine, but did not give the basis for the conclusion or a specific reference. Syntheses by Grün and Limpächer (20) and by Kabashima (22, 23), using aminoethanol, gave products sufficiently similar to natural cephalin to be considered by them as identical, and Rudy and Page (46) obtained, by special preparation from natural cephalin, a product which doubtless had only aminoethanol as its base. With cephalin the divergence of the elementary analysis from that calculated for the accepted formula has been much greater than has been the case with lecithin. The explanations offered for this discrepancy have been reviewed by MacLean and MacLean (39) and by Thierfelder and Klenk (55). Rudy and Page (46), by a careful fractionation of the mother liquor of ordinary cephalin, obtained a product which agreed with the theoretical composition. This product should correspond, however, to the material containing ethanolamine which Darragh and MacArthur (7) and MacArthur, Norbury, and Karr (38) found in their lecithin fractions, and not to typical cephalin, which has always been identified by its insolubility in alcohol.

Gray (17) presents a well-conceived survey of the differences between experimental and theoretical values for the analysis of cephalin. He concludes that the discrepancy may be accounted for by an additional group or groups low in carbon and hydrogen and rich in oxygen. He gives considerable evidence for the presence of such groups, but fails to identify definitely any such compound. He notes particularly that reduced cephalin is non-hygroscopic, while the reduced synthetic compounds of Grün and Limpächer (20) and of Kabashima (22, 23) were hygroscopic.

Christensen and Hastings (6), from a study of the electrophoresis and the titration of cephalin, concluded that both the titration behavior and the instability of cephalin point towards a structure other than the generally accepted one. Their observation that about 0.6 equivalent of potassium or sodium is bound per mole of cephalin may be significant.

Folch and Schneider (11) investigated the base by means of the nin-hydrin-carbon dioxide method and the periodate reaction, proving the presence of a hydroxyamino acid. The identification of glycolic acid in

the products from the reaction with ninhydrin and its isolation as the dimedon compound indicate that the amino acid present is serine. In confirmation, treatment with chloramine-T also yielded glycolic acid, which was isolated as the phenylosazone. Forty to 70 per cent of the nitrogen present in various samples was found to be amino acid nitrogen.

Folch and Schneider find 40 to 70 per cent of their cephalin preparation to consist of a cephalin with an amino acid base. Christensen and Hastings, using cephalin prepared in a very similar manner, find that about 0.6 equivalent of potassium or sodium is found per mole of cephalin. Hence it is entirely possible that it is the amino acid cephalin which binds the base and shows the buffering action believed by the latter authors to be characteristic of cephalin. If reference then be carried back to the discussion of Gray (17), and correction made for serine instead of aminoethanol, the calculated percentage of carbon comes somewhat closer to

TABLE 1
Analytical figures for cephalin (calculated and observed)

MATERIAL	C	H	N	P
	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Calculated for stearylinoylelcephalin, aminoethanol base.....	66.04	10.71	1.88	4.16
Calculated for stearylinoylelcephalin, serine base.....	63.96	9.97	1.77	3.93
Calculated for potassium salt of foregoing..	60.94	9.50	1.69	3.75
Observed values, average by Gray.....	60.41	9.79	1.67	3.59
Observed values, preparation by Folch and Schneider.....	60.0		1.605	3.89

that observed, but still the correspondence is not good. If, however, the serine cephalin be considered as combined with one equivalent of potassium, the agreement is almost perfect, as shown in table 1, copied after Gray with the addition of the data indicated. The analysis of the cephalin investigated by Folch and Schneider, which was purified in an exceptionally careful manner, is included. Gray found 12 per cent ash in his material, and since only a small part of this is explained as phosphorus, the assumption that a metal is present seems justified. Christensen and Hastings found 1.83 per cent potassium and 0.80 per cent sodium in the cephalin prepared according to the method of Levene and Rolf.

Folch and Schneider offer two explanations of their data: "There may be two cephalins preformed in the brain; or the ethanolamine-containing cephalin may be an artifact, originating post mortem by decarboxylation of the serine constituent." Figure 1 illustrates the decarboxylation suggested. Considering the wide divergence in properties of the two bases,

it seems unlikely that the name "cephalin" will be retained for these two compounds, and evidence seems to be gathering toward the support of the suggestion that the compound containing ethanolamine is a decomposition product.

Many workers have observed, and MacLean and MacLean (39) have discussed in particular, the fact that only when fresh tissue is obtained and dried quickly with avoidance of high temperatures is it possible to obtain a good yield of lecithin free from amino nitrogen. If the tissue is not fresh, or is not dried rapidly, a large amount of aminoethanol "cephalin" is found with the lecithin and is separated from it only with great difficulty. The obvious assumption would be that the lecithin is changed to cephalin, but this would require a demethylation of the lecithin, which scarcely seems possible.

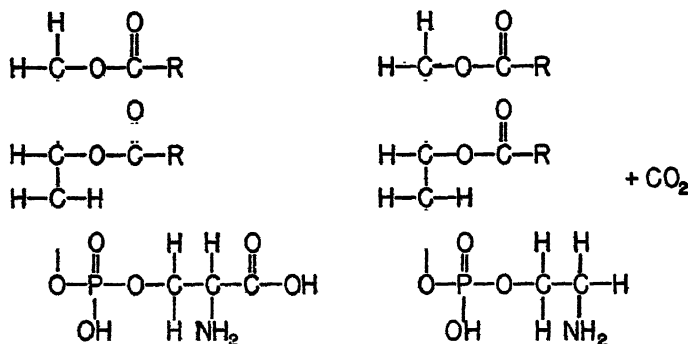


FIG. 1. Possible structure for cephalin

However, the anomaly clears up if we have lecithin and serine cephalin in the original tissue, since the serine cephalin precipitates readily and clearly with alcohol, and so is separated easily from lecithin. It is easy to understand that in old tissue, or during a slow drying, the serine could be decarboxylated. The aminoethanol "cephalin" thus formed is difficult to separate completely from lecithin, owing to its slight solubility in alcohol and considerable solubility in alcohol solutions of lecithin.

If cephalin does contain an amino acid, the carboxyl should account for the alkali-binding observed by Christensen and Hastings, and the zwitter-ion structure between the amino group and the phosphoric acid is still possible.

IV. SPHINGOMYELIN

Thudichum (56) was the first to isolate a white phospholipid material from a warm alcohol extract of brain tissue upon cooling. This white

Reichel conclude that natural sphingomyelin is probably a mixture of lignoceryl sphingomyelin and lignoceryl sphingomyelin fatty acid ester. Only in this way could they account for the presence of free fatty acids and lignoceryl sphingomyelin among the split products of enzymatic hydrolysis. As a result of their analysis, they give $C_{47}H_{97}N_2PO_7$ as the empirical formula for lignoceryl sphingomyelin. The observed percentage of nitrogen was 3.32 and that of phosphorus was 3.97, in comparison to calculated percentages of 3.36 and 3.73, respectively. In a continuation of their work, Thannhäuser and Reichel (55) synthesized various lignoceryl sphingosine fatty acid esters, sphingosine fats, and sphingosine amides, using various fatty acids. The successful synthesis of these compounds shows that both hydroxyl groups of sphingosine can be esterified.

Until 1929, sphingosine was considered to have seventeen carbon atoms with a double bond between the fourth and fifth. This was believed to be the case after Lapworth (30), Levene and West (35), and Levene (31) found that tridecyclic acid (C_{13}) was formed upon oxidation of sphingosine at the double bond. However, Klenk (24), and Klenk and Diebold (26) in 1939 obtained myristic acid and α -aminobutyric acid upon oxidation at the double bond with ozone and subsequent hydrolysis. Thus a chain of eighteen carbon atoms with an α -amino group and a double bond between carbon atoms 4 and 5 was indicated.

Sphingomyelin then is a phospholipid without glycerol as a foundation. Rather, the two hydroxyl groups and the one amino group on the terminal carbon atoms of sphingosine act as the linking groups to the attached radicals. The ceramide link to the lignoceryl acid radical is on the α -carbon atom, the cholinephosphoric acid radical is probably on the β -carbon atom esterified through the hydroxyl group, and the second hydroxyl group on carbon atom 3 may or may not be esterified to a fatty acid,—palmitic or possibly stearic. Carbon atoms 3 and 4 are asymmetric, and sphingomyelin is accordingly optically active. Recently, Thannhäuser and Benotti (51) have shown that sphingomyelin has a monomeric form rather than the polymeric form previously suggested (52). Their conclusions were based on results of molecular weight determinations made through boiling-point measurements, titration with acid, and analysis of the silver and platinum salts.

Christensen and Hastings (6) recently examined sphingomyelin in regard to its property to bind various ions. This property has been suggested in the past as due to zwitter-ion formation in the sphingomyelin molecule when in solution. Chain and Kemp (3) determined the isoelectric point for sphingomyelin by the electrophoretic method as being at or near pH 6.0. Fischgold and Chain (10) found that in benzene-alcohol solution sphingomyelin bound one equivalent of hydrogen ions in acid reactions but did not yield hydrogen ions in basic solution except to a very small extent, which

was apparently due to impurities. This seemed to favor the zwitter-ion form for sphingomyelin when in solution. Christensen and Hastings (6) titrated sphingomyelin electrometrically, and found that it did not show any buffering action over wide ranges of pH; they failed to find any appreciable combination of sphingomyelin with chloride or sodium ions.

V. PLANT PHOSPHOLIPIDS

Thierfelder and Klenk (55) gave a very adequate review of the literature on plant phospholipids to up 1930, and no important additions to our knowledge of their structure have appeared in the literature since that time. The presence of sphingomyelin has not been proven in plants, and Austin (1) particularly mentions its absence in yeast. Plant lecithins and plant cephalins corresponding closely to animal lecithins and cephalins have been prepared, but they appear throughout to be much more difficult to purify, especially as to freedom from sugars. This is generally considered to be a contamination rather than a structural part of the lipid, since several workers have obtained materials apparently free from sugar (see Schulze (47), Rewald (45), and Diemair, Bleyer, and Schmidt (9)). The phospholipids of cereals appear to be especially low in nitrogen and phosphorus. Those from barley, wheat, and oats, prepared by Diemair, Bleyer, and Schmidt, are typical in that they contain only about 1.0 per cent of nitrogen and 2.2 per cent of phosphorus, although they are apparently free from sugar. Calcium salts of the phosphatidic acids, as described by Chibnall and Channon (4), could explain low values for nitrogen, but no satisfactory explanation has yet been offered for the low amount of phosphorus found in many plant phospholipids. It is not yet clear what other impurities might cling so firmly to them as not to be removed by the methods used successfully with materials of animal origin.

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CONSTITUENTS OF FATS AND OILS AFFECTING THE DEVELOPMENT OF RANCIDITY¹

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I. INTRODUCTION

Like all organic materials, the fats are subject to deterioration. Most important of the degradative changes which may occur are those which lead to the condition known as rancidity. A fat may be described as rancid when it contains enough oxidation products or free short-chain fatty acids to provide organoleptic evidence of their presence. In those natural fats containing appreciable quantities of the lower fatty acids, hydrolysis induced by lipases derived from tissues or microorganisms is, in itself, sufficient to produce rancidity; oxidation may accompany such hydrolysis. However, spontaneous atmospheric oxidation, non-enzymatic in character, is by far the most common cause of rancidity. The "off" flavors and odors in various natural foods have been called rancid, pun-

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gent, tallowy, soapy, oily, ester-like (perfume or ketonic rancidity), metallic, musty, fishy, bitter, cardboard, and burnt. These descriptive terms are evidence of the complexity of the processes of rancidity.

The subject of oxidative rancidity in fats is obviously too extensive to be covered in its entirety here. No attempt will be made to discuss the proposed mechanisms, means of detection or measurement, or end products of the reactions resulting in rancidity. Several recent reviews covering various aspects of these fields of investigation are available (51, 54, 63, 80). Furthermore, the very broad subject of accelerators and inhibitors of these reactions has been limited to include only what is known about the natural constituents of fats and oils which affect their stability.

Since ethylenic linkages in the fatty acids are the points of attack by oxygen, it is obvious that the amount of unsaturation is among the factors which determine the susceptibility of fats to oxidation. Linoleic acid is oxidized more quickly than oleic acid (40, 79), and linolenic acid still more rapidly (47). A difference in the rate of oxidation was also observed between oleic acid and a mixture of oleic and linoleic acids (5), but oleic acid gave organoleptic evidence of rancidity sooner than the mixture, despite the higher peroxide value of the latter.

The molecular structure of the fatty acids also determines the ease with which a fat is oxidized (47). In polyethenoid acids the relative positions of the double bonds with respect to each other and in the chain are critical factors; thus, pseudo-eleostearic acid (conjugated) is more susceptible to oxidation than its isomer linolenic acid (non-conjugated) (44). The absorption spectra of edible oils indicate (56) that these contain traces of fatty acids with conjugate double bonds not accounted for by the amounts of recognized fatty acids present. The importance of this observation lies in the fact that these minute amounts of more reactive fatty acids may immediately give rise to active peroxides capable of initiating reaction chains. Morrell and his coworkers (57) and others (26) have made extensive studies leading to a differentiation between fat peroxides of differing reactivities. Little is known about the stability of synthetic simple or mixed glycerides.

It has long been recognized, however, that the stability of many fats and oils is *not* dependent primarily upon their relative saturation. Thus, sweet almond and sesame oils remain stable for much longer periods than other fats and oils of similar or higher iodine number (42). The extraordinary stability of wheat bran oil has lately been described (19). The first intimation that such stability could be attributed to naturally occurring antioxidants was suggested by Mattill (52), who confirmed the observation (2) that the addition of wheat germ oil to rat diets containing lard stabilized them against rancidity. Mattill and Crawford (55) later

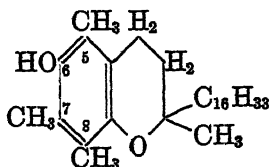
showed that the induction period of corn oil was markedly decreased by the various steps taken in its commercial production. Hilditch and Sleightholme (39) prepared purified fatty acids and glycerides from olive oil and found that they were very much more susceptible to oxidation than was the original oil. Thus, vegetable oils were shown to contain antioxidants which were progressively removed in attempts to obtain purified products. Even the most highly refined vegetable oils, however, still contained substances having demonstrable antioxygenic activity. Some of the constituents responsible for these reactions have been identified in the past few years, and an attempt will be made here to review the available information. Clear-cut conclusions are rare. The field is complicated by difficulties in assaying and in interpreting results. Substances which appear to be effective antioxidants under one set of conditions prove to be inactive in a different environment. Thus, for example, Evans (21) reported that potassium cyanide was an effective stabilizer for oleic acid when the oxidation was accelerated by the presence of cobalt oleate. However, in the absence of metallic catalysts, the cyanides are ineffective (61). Similarly, compounds effective in fats whose oxidation is accelerated by exposure to light may be inactive in the dark, and those suitable for use at room temperature may be useless in tests at elevated temperatures. Most unsatisfactory of all is the difficulty in duplicating antioxidant assays even under the most standardized conditions, due in all likelihood to a lack of appreciation of all of the factors involved in such measurements.

II. INHIBITOLS

Early attempts (9, 65) to concentrate the stabilizers occurring in various vegetable fats and oils utilized the methods employed in the preparation of vitamin E concentrates. Sterol-free unsaponifiable fractions obtained from wheat germ, cottonseed, and palm oils were effective antioxidants for animal fats and purified fatty acids and esters. No means could be found to separate the antioxidant from the vitamin, but since the two activities were not parallel in concentrates from different sources, it was assumed that the substances responsible for them were not identical. Those responsible for the stabilizing action were called inhibitols (67) to indicate that they were effective by virtue of hydroxyl groups which, as in the phenolic antioxidants, became ineffective on esterification. With the isolation of vitamin E and the demonstration that there were several tocopherols (22), the earlier confusion was resolved; α -tocopherol is more effective as vitamin E than β - or γ -tocopherol, whereas the latter are the more effective antioxidants (64). Although the tocopherols appear to be the principal antioxidants in certain vegetable oils, the existence of other

inhibitols, some of which may not appear in the unsaponifiable fraction, is by no means excluded. Among these are certain vitamin K compounds (28), especially in their reduced form, as well as sesamol, gossypol, and others.

The tocopherols contain the aromatic ring and a free hydroxyl group



α -Tocopherol or 5,7,8-trimethyltolcol

(β -tocopherol is 5,8-dimethyltolcol; γ -tocopherol is 7,8-dimethyltolcol (20))

characteristic of known phenolic inhibitors (62). Ethers and esters, except for the allophanates (64), are inactive as antioxidants. A study of the parent chromans and related substances has shown (28) that the heterocyclic oxygen is as essential to the antioxygenic action of the tocopherols as is the second hydroxyl group in hydroquinone or catechol. The introduction of successive methyl groups decreases the stabilizing action of chromans (and tocopherols), just as it does that of hydroquinone.

The tocopherols can be concentrated in the residue obtained from the distillation of methyl or ethyl esters prepared from vegetable oils (68), but are found in the first fractions when the oils themselves are subjected to molecular distillation (16). Riemenschneider *et al.* (70) have described an unusual stability in the first fraction from such a distillation of *refined* cottonseed oil, while Fawcett (23) has shown that antioxidants are concentrated in both the first fraction and the residue during the molecular distillation of *crude* cottonseed oil.

Although inhibitol fractions and tocopherols were found to be effective stabilizers for animal fats, they had little, if any, antioxygenic value when added to the vegetable fats from which they were obtained (68). This paradoxical result rests only in part on the use of too small amounts, proportionately, for test purposes; no adequate explanation for the phenomenon will be forthcoming until more is known about the mechanism by which tocopherols inhibit oxidation. Preliminary observations (27) indicate that the oxidation of tocopherol during the induction period follows slightly different paths in animal and in vegetable fats. Nakamura (58) has reported that relatively large amounts (0.5 to 1.0 per cent) of the unsaponifiable fraction from soybean oil stabilize soybean oil slightly.

Various other substances, however, do act as effective stabilizers for vegetable fats, among them certain inorganic and organic acids (17, 33, 68) most of which have little if any stabilizing effect on animal fats. They

are relatively insoluble in fats and are not fat constituents as such, but they may and do occur in association with fats under natural conditions, perhaps in amounts undetectable by ordinary chemical means. Citric and ascorbic acids are present in milk, and phosphoric acid, in the form of phospholipid, is widely distributed in fats of animal and plant origin. Since these acids stabilize vegetable oils containing inhibitols but do not stabilize animal fats (68), their action is described as synergistic with the inhibitols.

The chemical mechanism of this synergism has not been investigated in any detail except with ascorbic acid. The effect of this acid in reducing the susceptibility of milk fat to oxidative deterioration was first indicated by experiments in which it was fed to cows with satisfactory results (10), but even before that time its capacity to act as a synergist with inhibitols had been observed (61). Its value in increasing the keeping quality of milk fat has been the subject of several recent inquiries (45), and its use as a stabilizer in mayonnaise has been described (31). Ascorbic acid is an excellent stabilizer of tocopherol (41). A recent study (30) has indicated the nature of this action. Successive determinations of tocopherol in autoxidizing fat substrates during the progress of the induction period showed that it disappeared rapidly at first, and later more slowly, and that by the end of the period the tocopherol had all been oxidized to tocoquinone and beyond. This was also true when ascorbic acid was present, but the induction period was greatly prolonged and, again, tocopherol had disappeared by the end of the induction period. Ascorbic acid also slowly disappeared, but animal fat substrates containing ascorbic acid without tocopherol became rancid while most of the ascorbic acid was still present (27). The oxidation of tocopherol appears to be a two-step oxidation, the first (reversible) step being the formation of a phenoxyl radical (29). The apparent oxidation potential of tocopherol is 0.656–0.597 volt; that of ascorbic acid is much lower, $E_0 = 0.390$ volt. It would appear, therefore, that ascorbic acid is not appreciably oxidized by fat peroxides and does not prevent their formation, but it retards the oxidation of tocopherol, which in turn temporarily prevents the oxidation of fat and the initiation of reaction chains.

The stabilizing action of phosphoric acid is also confined to those fats and oils which contain tocopherol or related inhibitols; by itself phosphoric acid has little antioxygenic effect on animal fat substrates (68). Studies by Golumbic (27) on the oxidation of tocopherol in the presence of phosphoric acid indicate that phosphoric acid favors ring closure of toco(hydro)-quinone with re-formation of tocopherol, a cyclization that has become well known in the chemistry of vitamins E and K.

Obviously, the intimate chemistry of the synergism between inhibitors

offers many interesting problems of fundamental nature and practical importance.

III. PHOSPHOLIPIDS

The antioxidant properties of crude lecithin preparations were discovered by Bollman (8) and described briefly by several other investigators (21, 46). Olcott and Mattill (66) showed that the various commercial preparations were more effective in vegetable oils than in animal oils or purified fatty acids or esters, although marked activity could be demonstrated in the latter substrates, if tocopherols or other phenolic inhibitors were added (68). Purified lecithin was inactive, but the activity appeared quantitatively in the cephalin fraction. Hilditch and Paul (38) found that highly purified soybean phosphatides were inactive in olive oil esters. Similarly, Diemair and Fox (14) found that purified oat oil phosphatides were not antioxygenic, although the complex system from which the phosphatide had been separated (a protein-phosphatide complex (15)) was supposedly the most active factor in producing the antioxidant effect of oat oil. The reported inactivation of lecithin by heating above 65°C. (21, 73) has not been confirmed (61).

In view of the somewhat conflicting results and the recent suggestion that a considerable part of the cephalin fraction may contain serine rather than colamine (24), a reinvestigation of the problem would be timely. If the acid reaction of cephalin is due to the carboxyl group of serine, the hypothesis that the antioxidant activity can be ascribed solely to an ionizable phosphoric acid (66) may be subject to revision.

IV. CAROTENOIDS

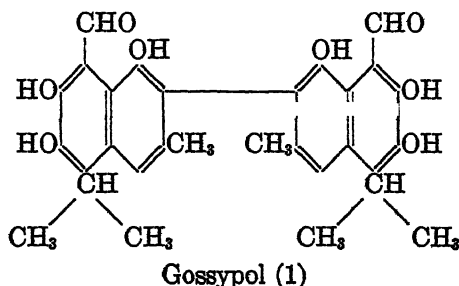
The effects of carotenoids on the oxidation of fats and oils have been studied in several laboratories. With the oxygen absorption technique applied to lard or mixtures of lard and cod-liver oil, it was found that carotene, xanthophyll, and lycopene acted as prooxidants in decreasing the length of the induction period (9, 62). Franke (25) found that carotene and xanthophyll were prooxidants for oleic and linoleic acids but were inactive in linseed or olive oil. Newton (59) concluded that carotene was an antioxidant, because fatty extracts of carotene-containing sources such as paprika, alfalfa, and palm oil acted as antioxidants in lard. Inasmuch as the effect was enhanced by bleaching the carotene, the activity of the extracts has been interpreted as indicating the presence of non-carotenoid antioxidants, presumably of the inhibitol type (67). Thus, the presence of easily oxidizable carotenoids in fats and oils might appear to be presumptive evidence for the presence of protecting antioxidants. Täufel and Müller (77) recently recorded that xanthophyll possesses only slight prooxygenic activity in chicken fat and butterfat.

The occurrence of synergistic effects is suggested by Franke's (25) observations that carotene and xanthophyll in the presence of hemin were proöxygenic for olive and linseed oils, whereas, in the presence of pyridine, marked antioxidant activity was noted.

The action of vitamin A may be assumed to be similar to that of the carotenoids, although the effect of the pure compound on the induction period of fats has not been described. Esters of vitamin A are more stable toward oxidation than is the free vitamin (37) and would presumably be less likely to act as proöxygens.

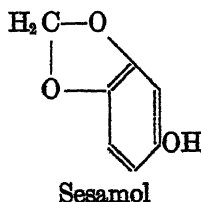
V. GOSSYPOL

Crude cottonseed oil may contain from traces to 1.5 per cent (expeller oil) of gossypol. Mattill (53) first recorded and Royce (71, 72) confirmed the marked antioxidant activity of this substance. Royce concluded that gossypol was the most effective antioxidant in crude cottonseed oil and was responsible for the better keeping quality of crude over refined cottonseed oil. The multiple phenolic groups of gossypol are sufficient to account for its activity.



VI. SESAMOL

Sesame oil, like other vegetable oils (36, 52, 60), has the property of imparting stability to other fats when added in small amounts. The unsaponifiable fraction contains a compound, sesamol, which Böeseken, Cohen, and Kip (7) consider a glucoside of sesamol, the phenolic sub-



stance responsible for the Villavecchia and Baudouin color tests for sesame oil. Sesamol is an effective inhibitor in fats and oils (61). Perhaps the gradual liberation of sesamol from sesamolol creates a particularly favor-

able environment for preventing oxidation. The distillate secured during the deodorization of hydrogenated sesame oil is antioxygenic (35) and possibly contains sesamol. Sesamin (7), a closely related compound and one more easily isolated, has no antioxidant properties (61).

VII. CHLOROPHYLL

The importance of chlorophyll in accelerating the oxidation of oils exposed to light has been emphasized by Coe (11) and Täufel and Müller (77). The addition of chlorophyll is said to reduce the induction period markedly, and rancidity occurs at very low peroxide values (11). Pure chlorophyll is a more effective prooxidant than crude preparations (77). Although most crude oils contain chlorophyll, refined oils contain only traces, if any (6), and it has been pointed out that the oils from which the chlorophyll has been removed are less stable than those containing maximum amounts (6). Presumably, the effect of antioxidants is more pronounced than that of the prooxidant chlorophyll in these reactions. Under conditions of ordinary in-door illumination, added chlorophyll does not affect the induction period of lard (27, 61).

Coe (13) has devised a method for determining relative stability, based upon the ability of fresh oils to quench the red fluorescence of magnesium-chlorophyll. As the oils become oxidized, the quenching effect decreases. Bickford, Anderson, and Markley (6) have suggested that dissolved oxygen in the oil under test is the responsible agent. However, the quenching action is not altered by degassing in a high vacuum, and the substance or substances responsible for the blue fluorescence and quenching can be concentrated in the unsaponifiable fractions (lard and cottonseed oil) (61). Hence, it appears more likely that the fluorescence is connected in some way with the antioxidants or other oxidizable constituents in the unsaponifiable fractions, the concentration of which decreases during the induction period. Pure α -tocopherol fluoresces with a somewhat darker blue than the crude unsaponifiable fractions, but both possess the property of quenching the red fluorescence of chlorophyll.

VIII. WATER

The presence of water in fats may modify the reactions leading toward rancidity in several ways. The evidence as to the effect of moisture on the rate of oxidation of pure fats leads to the conclusion that water probably has a slight retarding effect (51).

The presence of moisture in fats, however, permits the growth of organisms, some of which possess powerful enzyme systems capable of accelerating glyceride hydrolysis (lipases) and oxidation (oxidases or lipoxidases). Jensen and Grettie (43) have shown that as little as 0.3 per cent

of water in fats is sufficient to permit the growth of bacteria and molds. Insofar as fats or oils are susceptible to this type of spoilage, substances which inhibit such growth might be classed as antioxidants. Added phenols in such systems would thus play a double rôle, that of inhibiting bacterial growth as well as protecting the fats from oxidative deterioration.

Lipoxidases are known to be fairly widely distributed in the animal and vegetable kingdoms, having been identified in soybean, wheat germ, peanut, etc., and in adipose and muscle tissue (50, 51). Presumably, these are effective only in the presence of water. Soybean lipoxidase also appears to be indirectly responsible for the bleaching of carotene in unsaturated fats (76), a reaction which had been assumed to be the function of a separate enzyme (75).

Lea (49) studied the effect of numerous water-soluble compounds on the oxidation of lard in contact with an aqueous phase and found that citric and related acids and several amino acids were the most effective inhibitors. The obvious complexity of such systems makes interpretation difficult.

Hilditch and his coworkers (3, 39) found that the induction period of olive and linseed oils was markedly reduced by treatment with boiling water for 3 hr. Carbon dioxide was bubbled through the mixtures to prevent oxidation. Inasmuch as no activity could be recovered from the aqueous layer, the antioxidants involved were destroyed by such treatment. These observations have been duplicated for cottonseed and sesame oils (61). There is as yet no definite clue as to the nature of the compounds affected. In the case of cottonseed and sesame oils, the treatment not only decreased the length of the induction period, but also changed the reactions of the oils toward the two types of inhibitors. The acid inhibitors were no longer as effective, while some activity could be demonstrated with inhibitol concentrates.

Elder (19) has described a decrease in the induction period of wheat germ oil following a cook with brine, presumably due to the leaching out of antioxidant.

IX. MISCELLANEOUS SUBSTANCES

The antioxidant activity of unsaponifiable fractions of oils and fats is found in the non-sterol constituents. Purified sterols from a number of sources were found to be uniformly inactive (53, 58). The occasional references to antioxygenic sterols usually involve impure preparations of sterols.

The concentration of metal salts in oils and fats is almost vanishingly small, yet, since these substances have been shown to be such powerful prooxidants, some mention of their effects should be included. For ex-

ample, the processing of fats or fatty foods in contact with metal is known in some cases markedly to accelerate oxidative decomposition. Copper and iron are the common offenders. Lea (51) has summarized the information available on this subject.

Aside from the natural constituents of fats and oils which affect their susceptibility toward oxidative rancidity, there occur in nature numerous compounds and materials which, upon addition to fats and oils, act as antioxidants. Gum guaiacum (34) and gallic acid (68) are examples. Cereal and oil-seed flours have been found to act as antioxidants (69), but only part of the antioxidant activity can be extracted with fat solvents (61). Hilditch *et al.* (32, 38) have described antioxidant fractions extractable from oil-seed meals after digestion with organic acids. These possessed marked reducing power and contained small amounts of phosphorus and nitrogen, and their activity could be destroyed by exposure to traces of hydrogen chloride. Greater yields were obtained from the meals than from the oils themselves.

Coe (11, 12) has claimed that catalase preparations are more effective antioxidants in the light-catalyzed oxidation of vegetable oils than any others except pyrogallol. However, Täufel and Müller (78) found that the antioxidant activity of liver catalase preparations not only did not parallel the enzymic activity but persisted after the removal of the catalase. The antioxidant activity of cottonseed meal has been found not to depend on the catalase present (61), and highly purified catalase preparations were inactive as antioxidants in lard (not light-catalyzed) (27). The effect of catalase and the rôle of hydrogen peroxide in rancidity reactions thus require further investigation. Coe (11) was unable to detect catalase activity in oils or fats.

The "soft" fat of animals given oil-containing diets is more susceptible to oxidation than the fat laid down on rations not containing such oils (4, 48). Butterfat from summer pasturage cows is more unsaturated than that obtained during the winter, but of two samples of equal iodine number, the summer butter is the more stable (74). Much remains to be learned about the influence of ration constituents upon the susceptibility of body and milk fats to oxidation.

Elder (17) has found that the oil of roasted coffee has three times the induction period of that obtained from the original green beans, presumably owing to the synthesis of an antioxidant, possibly pyttrole, during roasting.

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SYNTHETIC FATTY ACID GLYCERIDES OF KNOWN CONSTITUTION¹

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I. INTRODUCTION

A reasonably clear picture of the chemical constitution of natural fats and oils is basically dependent upon our knowledge of the molecular structure of the individual components. Because of the many difficulties inherent in isolating the individual glycerides from natural products, only moderate progress has been made in that direction. No doubt such progress will be greatly accelerated by the newer techniques that are being developed, such as molecular distillation in conjunction with fractional crystallization and solvent partition. Nevertheless, the postulated structures of nearly all isolated products will need to be checked against the evidence provided by synthesis, because nearly all of the constituent fatty acids occur as mixed triglycerides which can exist in either of two or more stable isomeric forms. The work in our laboratory has been chiefly of two types: (a) studies of satisfactory methods of synthesis, and (b) studies of relationships

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between the structure and properties of known isomers.² We feel that the synthesis of isomeric mixed triglycerides by reliable methods offers a valuable approach to the study of natural fats and oils, in that such work provides physical and chemical data for identification purposes and also permits correlations between structure and properties from which one can outline procedures for separating anticipated mixtures from natural products.

II. EARLY WORK ON MIXED TRIGLYCERIDES

Although Chevreul (24), in 1823, concluded that natural fats were primarily glycerol esters of oleic, palmitic, and stearic acids, it remained for Berthelot (13) to point out that they were probably composed of mixed triglycerides rather than mixtures of such simple glycerides as triolein and tristearin. Fritzweiler (41), and later Klimont (76, 77, 78), verified Berthelot's conclusion by the isolation of oleodistearin from cacao butter, oleodistearin and oleodipalmitin from tallow, and steardipalmitin from duck and goose fat. Hansen (53) and Kreis and Hafner (80) obtained palmitodistearin from mutton and beef tallow, and Bömer (16) and associates obtained dipalmitostearin and palmitodistearin from mutton tallow. From 1907 to 1938 Bömer and associates (17, 18, 19, 20, 21, 22) continued actively in the isolation of component glycerides from fats by fractional crystallization methods, investigating especially the composition of mutton tallow, lard, coconut oil, palm kernel fat, nutmeg butter, and babassu fat. From goose fat, Amberger and Bromig (4) separated palmitodistearin, steardipalmitin, and steardiolein.

All of these investigations gave support to the conclusion that fats and oils consist mainly of mixed triglycerides, but there was essentially no evidence relative to the configuration of the isolated products. Investigations since about 1927 by T. P. Hilditch (7, 62, 63, 64, 65, 66, 72) and associates, including progressive hydrogenation and oxidative researches, served to confirm and extend the view that fats are very complex mixtures of mixed triglycerides. In a few instances, the authors also assigned a definite configuration to the products isolated. Hilditch and Saletore, for example, reported the isolation of β -oleo- α , α' -distearin. The natural triglyceride upon oxidation gave a product which did not depress the melt-

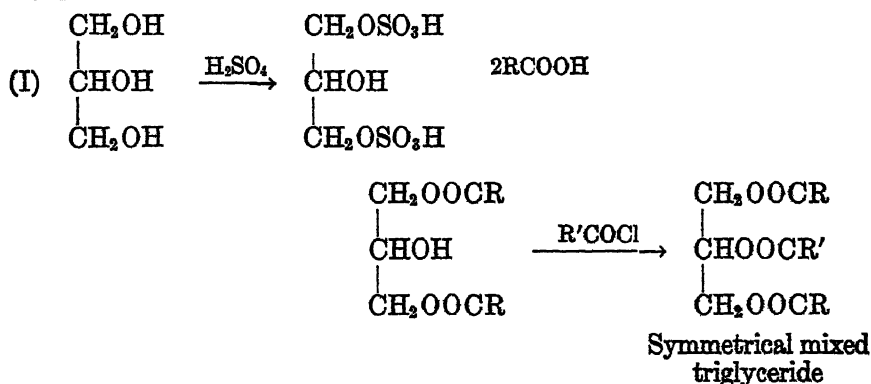
² We are indebted to The Buhl Foundation for grants in support of our work during the past three years, and to Professor G. Stegeman, Dr. T. H. Clarke, and Dr. H. E. Longenecker for their coöperation. The scope of our work has been accordingly extended to include the following: (c) thermal studies (specific heats and heats of combustion) of known synthetic isomers; (d) synthesis with unsaturated fatty acids; and (e) studies of the nutritional factors that markedly affect the composition of fats.

ing point of β -azela- α , α' -distearin synthesized from α , α' -distearin. There was evidence, too, that the same symmetrical triglyceride could be found in a number of seed fats. Hilditch and Stainsby (71) obtained similar evidence that palmitodiolein, a constituent of many fats, had the beta or symmetrical configuration. Their conclusions were supported by the additional fact that palmitodistearin, obtained by hydrogenation of the oleic acid esters, melted at a temperature close to that of the synthetic symmetrical triglyceride. Other evidence relative to the specific configuration of mixed triglycerides has been obtained by Hilditch and Paul (68), Hilditch and Lea (67), and Banks, Dean, and Hilditch (6).

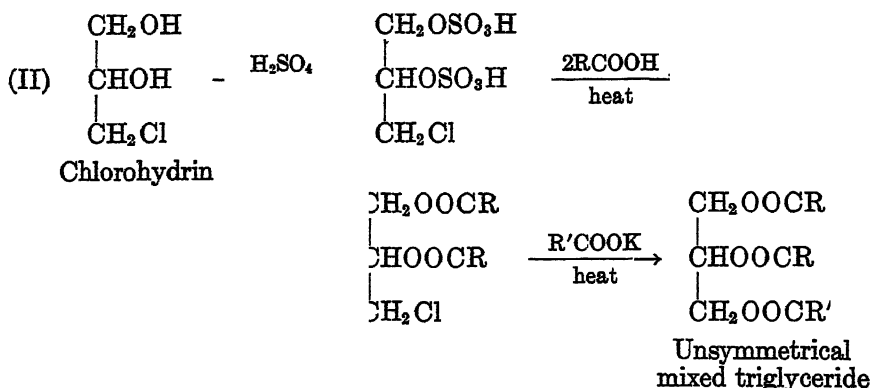
III. DEVELOPMENT OF RELIABLE METHODS OF SYNTHESIS

Berthelot's (14) method of heating fatty acids with an excess of glycerol was perhaps the first laboratory procedure used for the synthesis of glycerides. By heating stearic acid with glycerol for a period of 20 hr. at 200°C. in a sealed tube, he obtained a monostearin and, by further heating, a distearin. Although the first two products were probably impure, he later obtained a synthetic tristearin corresponding to natural tristearin by heating monostearin with a large excess of stearic acid. Simple triglycerides were obtained by Garner (42) in almost theoretical yield by heating equivalent quantities of fatty acid and glycerol at 200°C. in an atmosphere of carbon dioxide. Although these and many other methods (57, 84, 97) are suitable for the preparation of simple triglycerides, they are of no practical value for the synthesis of pure mixed triglycerides.

The methods available for the preparation of mixed triglycerides before 1920 were chiefly those of Grün and associates (46, 48, 50). The preparation of a symmetrical mixed triglyceride by the action of an acyl chloride on glycerol disulfuric acid ester was carried out as indicated by the following procedure:

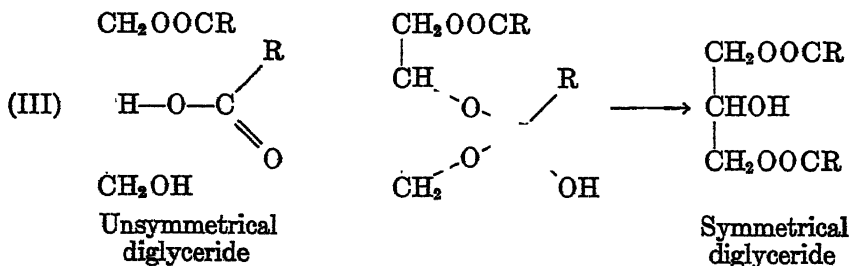


The supposed unsymmetrical triglyceride was prepared from the disulfuric ester of monochlorohydrin and the potassium salt of a fatty acid:



Grün and Limpächer (47) also employed the reaction methods of Romberg (87), Guth (52), and Kraft (79), using halohydrins with the sodium or potassium salts of the fatty acids, but discovered that glycide formation resulted in the production of other glycerides, diglyceryl esters, and unesterified fatty acids. Other investigators (5, 32) found that, because of (a) the temperature at which the reactions were carried out, (b) the uncertainties regarding the structure of the intermediate halohydrins and disulfuric esters used, and (c) the possibilities of rearrangement during the course of the reaction, the above methods were not satisfactory.

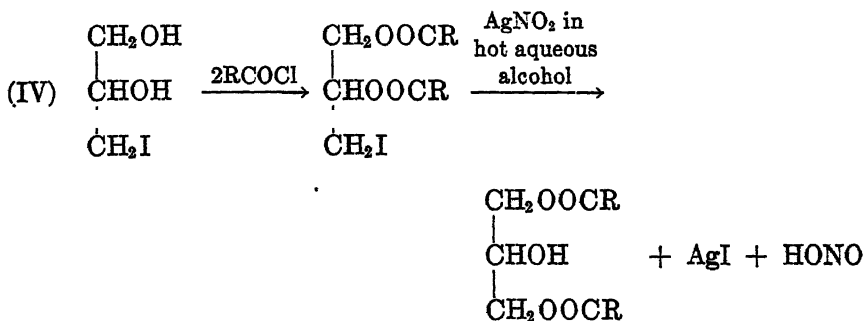
Fischer suggested that shifting of acyl groups might occur at high temperatures and then demonstrated that it actually occurred when he prepared mixed triglycerides by the methods of Grün. He postulated a mechanism to account for the rearrangement, based upon the production of a dioxolone derivative formed by the migration of a hydrogen atom on the carbon atom adjacent to the carbonyl group. The rearrangement, leading to the mistaken identification of β -monosubstituted and α,β -disubstituted products, may be indicated as follows:



Hibbert and Grieg (59) reported the isolation of an intermediate dioxolone derivative in their attempts to prepare the monotrishloroacetate of

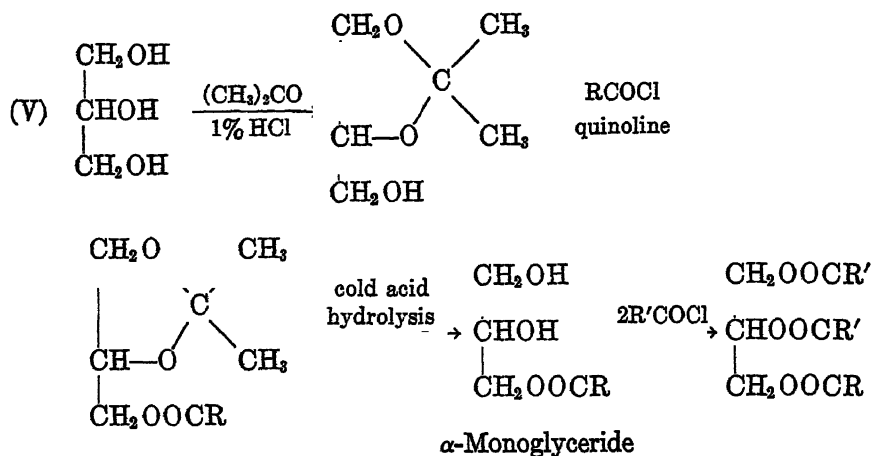
ethylene glycol. The tendency for the formation of a stable ring is apparently enhanced by increasing the polarity of the acyl group. Thus it is entirely plausible that the shifting of acyl groups from the beta to the alpha position may occur through the formation of a cyclic isomer.

The preparation of mixed triglycerides depends primarily upon (a) an initial selection of stable and pure starting compounds, (b) the preparation of pure mono- and di-substituted derivatives of glycerol, (c) the use of reaction conditions that do not cause unexpected shifts in position or replacement. The use of impure halohydrins as starting compounds and the above outlined (III) beta-to-alpha shifts of acyl groups have been frequent causes of error. Recognizing the need for intermediates of definite configuration, Fischer and associates (39, 40) developed methods for preparing α -monoglycerides and symmetrical diglycerides and, from these intermediates, symmetrical and unsymmetrical mixed triglycerides. Alival, α -iodohydrin, had been used for the preparation of supposed α,β -diglycerides and thence unsymmetrical triglycerides, but Fischer succeeded in showing that the resulting diglyceride had undergone a beta-to-alpha shift:

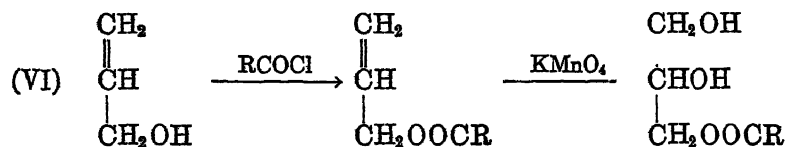


For the preparation of an α -monoglyceride (V), glycerol was first condensed with acetone to give 1,2-isopropylideneglycerol (acetoneglycerol), the structure of which has been carefully verified by Hibbert and Carter (58). Irvine, McDonald, and Soutar (74) had shown previously that acetone in the presence of acid condenses with compounds containing vicinal hydroxyl groups. Their methylated 1,2-isopropylideneglycerol, prepared from the condensation product of acetone and glycerol, hydrolyzed to an alpha methyl ether identical with that obtained from allyl iodide. Hibbert and Carter were unable to obtain the 1,3-isopropylideneglycerol by direct condensation but did isolate it by alkaline hydrolysis of 2-benzoyl-1,3-isopropylideneglycerol. Fischer esterified 1,2-isopropylideneglycerol with an acyl chloride in the presence of cold quinoline and then removed the acetone by cold acid hydrolysis, giving an α -monoglyc-

eride. The latter was then reesterified by a second acyl halide to yield the unsymmetrical triglyceride. The reactions were as follows:

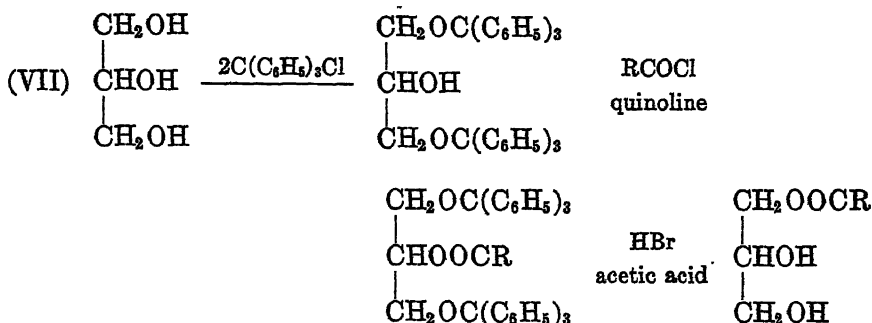


The structure of the monoglycerides prepared by this method was verified by Grün and Limpächer (47) by recondensation with acetone, and by Fairbourne and associates (33, 35) by the preparation of identical compounds, both from the allyl esters (VI) and by condensation with alpha monosodium glyceroxide (34, 37):



Abderhalden and Eichwald (1) contributed evidence to support the assigned structure by means of molecular rotation studies, and recently Hilditch (69) furnished additional evidence concerning the configuration on the basis of oxidation by lead tetraacetate. The synthesis of α -monoglycerides by the method of Fischer has repeatedly been found satisfactory by other investigators (3, 15, 26, 28, 83, 86, 95) for the preparation of unsymmetrical triglycerides.

Daubert and King (28) obtained good yields of pure α -monoglycerides also by the esterification of 1,2-benzylideneglycerol, followed by acid hydrolysis. The 1,2-isomer is frequently obtained as a predominant by-product when the 1,3-isomer is prepared for beta esterification. Jackson and King (75), studying the trityl ether methods discussed by Helferich and Sieber for preparing β -monoglycerides, found that, because of the shifting of acyl groups, α -monoglycerides were isolated:



These observations were verified by Verkade (98) in extensive studies of the use of trityl derivatives for the synthesis of glycerides.

Schuette and Hale (88) prepared α -monoacetin and α -monobutyryn by the direct esterification of glycerol in the presence of syrupy phosphoric acid. Robinson, Roche, and King (86) found that α -monoacetin and α -monobutyryn could not be obtained satisfactorily by hydrolysis of the esterified 1,2-isopropylideneglycerol. Young and Black (101) obtained α -monolaurin by heating trilaurin with glycerol and trisodium phosphate under anhydrous conditions. Other direct esterification methods include those of Bellucci and associates (8, 9), Gianoli (43), and Hilditch and Rigg (69).

Fischer and Baer have recently developed an excellent method of preparing optically active 1,2-isopropylideneglycerol and thence the corresponding α -monoglycerides (optically active) and mixed triglycerides (optically inactive), starting with the respective *d*- and *l*-mannitols. Since their work is treated in detail in another paper³ in this Symposium, further reference to it will not be made here.

Relatively little work has been done with mixed triglycerides containing unsaturated acids. There is evidence, however, that a more comprehensive study of this field will be made in the near future, in part because the techniques of obtaining pure unsaturated acids have been greatly improved. Carter and Malkin (23) have reported physical data for a few unsaturated triglycerides and Amberger and Bromig (3), in 1922, prepared α -monoolein from which a few monounsaturated unsymmetrical triglycerides were prepared. Recently, Black and Overley (15) reported the synthesis of α -monolinolein from tetrabromostearoyl chloride and acetoneglycerol. The esterified acetoneglycerol was hydrolyzed in cold acid solution and then debrominated with zinc and alcohol to yield the unsaturated α -monolinolein.

³ Chem. Rev. 29, 285 (1941).

A summary of the best published data for some of the triglycerides that have been isolated from natural products is given in table 1, together with

TABLE 1

Synthetic and natural mixed triglycerides: a comparison of melting-point data for typical compounds

GLYCERIDE	MELTING POINT OF SYN- THETIC PROD- UCT	ISOLATED NATURAL PRODUCTS*				
		Structure	Melting point	Author	Source	Year
	°C.		°C.			
1-Palmitodistearin . . .	64	(unsym.)	68.5	Bömer	Lard	1913
2-Palmitodistearin . . .	68	(sym.)	63.5	Bömer	Goose fat	1922
			68.2	Amberger	Cocoa fat	1924
			63.5	Kreis <i>et al.</i>	Tallow	1903
			66.2	Kreis <i>et al.</i>	Lard	1903
			62.8	Amberger	Hydrogenated butter fat	1913
			63.6	Bömer	Mutton tallow	1909
1-Stearodipalmitin . . .	62.5	(unsym.)	57.5	Amberger and Bromig	Goose fat	1921
2-Stearodipalmitin . . .	68	(sym.)	63.0	Amberger and Bromig	Goose fat	1921
			57.5	Bömer	Mutton tallow	1909
			58.2	Bömer	Lard	1913
			55	Hansen	Beef tallow	1902
			58	Amberger	Butterfat	1913
			59	Keimont	Goose fat	1909
1-Palmitodimyristin . .	54		45.2	Bömer	Palm kernel oil	1924
2-Palmitodimyristin . .	60		45.1	Bömer	Coconut oil	1920
1-Myristodilaurin	43		34.9	Bömer and Hüttig	Babassu fat	1938
2-Myristodilaurin	50		33	Bömer	Coconut oil	1920
			33	Bömer	Palm kernel oil	1924

* Specific structure not assigned unless indicated.

comparable data based on synthetic glycerides of known structure. Perhaps the need for reliable reference data based upon synthetic products of

known constitution will be indicated by the record. Bömer, Amberger, Klimont, and their collaborators are generally credited with having done the most thorough work that has been published relative to the separation of individual triglycerides from natural fats.

A number of mixed glycerides containing oleic and palmitic, oleic and stearic, or oleic, palmitic, and stearic acids have been isolated, but the recorded data are very inconsistent. Now that pure unsaturated fatty acids are becoming available for making intermediates, there is evident need for synthetic work with such series, in even greater degree than for the saturated series.

The data gained from the study of synthetic glycerides in recent years have been reasonably consistent in their indication that the symmetrical mixed triglycerides, compared to the unsymmetrical isomers, are less soluble, have higher melting points, and have higher indices of refraction. There is also some evidence that the symmetrical types are more common in occurrence (reference 23, page 181 *et seq.*), though a part of the evidence may be the result of their lesser solubility. Only a small amount of work has been accomplished relative to the synthesis of isomeric glycerides containing three fatty acids.

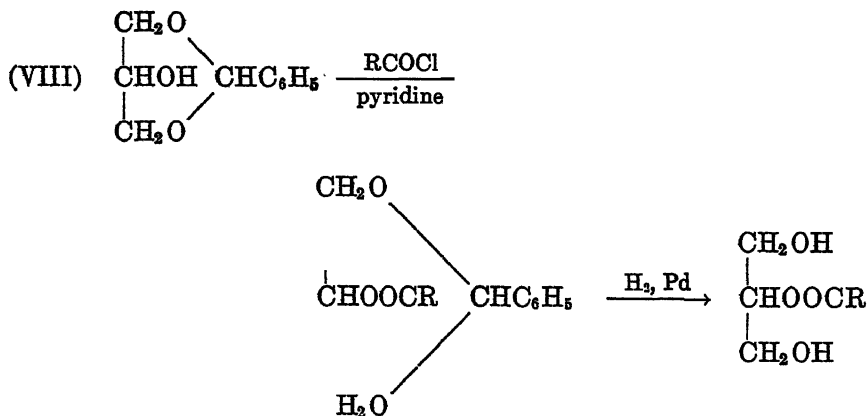
IV. MONOGLYCERIDES OF THE BETA TYPE

Many studies have also been directed toward finding suitable methods of preparing the β -monoglycerides and the α, β -diglycerides. Grün (50) supposedly prepared a beta ester of glycerol by treating 1,3-dichlorohydrin with an acyl chloride and subsequently removing the chlorine atoms. He also prepared what he believed to be β -monolaurin from β -monochlorohydrin and the potassium soap of the fatty acid. Both of these reactions have been investigated by Fairbourne (34, 35) and have been found to be of little value for the preparation of β -monoglycerides. Grün originally differentiated the isomeric monoglycerides by reaction with phenylurethan, but Fairbourne proved that the α - and β -monolaurins prepared by Grün's method gave identical diphenylurethans. Furthermore, he found that the β -monochlorohydrins used by Grün were mixtures of α - and β -isomers. Hibbert, Whelen, and Carter (61) contributed a valuable reference compound for identifying glycerides by preparing the beta methyl ether of glycerol and differentiating it from the alpha monomethyl ether obtained by Irvine, McDonald, and Soutar (74). They, as well as Fairbourne (31, 36), found no evidence to indicate a shift of methoxyl groups under common conditions of reaction, but Gilchrist and Purves (44) reported finding evidence of migration (unconfirmed). Helferich and Sieber (55, 56) undoubtedly obtained true beta aromatic esters of glycerol. Acid hydrolysis of 2-benzoyl-1,3-dinitritylglycerol and 2-*p*-nitro-

benzoyl-1,3-ditritylglycerol, prepared from 1,3-ditritylglycerol, yielded the β -monobenzoate and β -*p*-nitrobenzoate. Although they prepared the analogous aliphatic beta esters of ditritylglycerol, they did not succeed in preparing the derived simple fatty acid esters of glycerol. Jackson and King (75) prepared the β -palmityl- and β -stearyl-ditritylglycerols and found that acid hydrolysis, even at 0°C., gave good yields of the rearranged α -monopalmitin and α -monostearin. In fact, the method is one of the best for preparing pure α -monoglycerides.

Bergmann and Carter (12) first prepared a true β -monoglyceride of a fatty acid. Their method was based upon the catalytic reduction by means of hydrogen and palladium, in neutral solution and under pressure, of an esterified 1,3-benzylideneglycerol (VIII). Fischer (38), as early as 1894, had studied the products resulting from the condensation of benzaldehyde and glycerol, and later the same reaction had been investigated by Irvine, McDonald, and Soutar (74). Hibbert and Carter (58) succeeded in separating the two possible isomers by differences in solubility and identified the structures by methylation and hydrolysis.

Bergmann and Carter prepared β -monoacetin, β -monopalmitin, and glycerol β -monobenzoate by catalytic reduction of the corresponding beta esters of 1,3-benzylideneglycerol according to the following reactions:

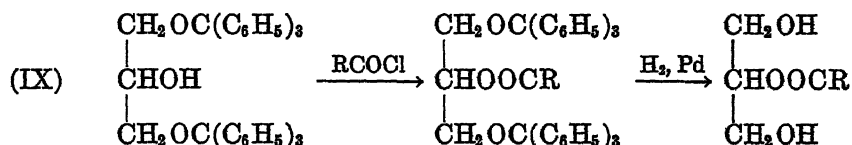


Stimmel and King (89) studied the method and the stability of the resulting esters further, including the preparation of the β -monoglycerides of capric, lauric, myristic, and stearic acids. Although the melting point of each of the beta esters was lower than that of the unsymmetrical isomer, the melted beta esters did not undergo rearrangement rapidly except when the reaction was catalyzed by hydrogen or hydroxyl ions. The beta esters were more soluble in organic solvents than the respective isomeric α -monoglycerides, in contrast to the lesser solubility of the symmetrical mixed

triglycerides and the aromatic acid glycerol esters of the beta type. Proof of structure of the β -monomyristin was obtained by converting it to the known 2-myristyl-1,3-distearin (86) by treating the beta ester with two moles of stearyl chloride under controlled conditions.

Hibbert and Carter (58), in testing the preparation of aromatic beta esters by hydrolysis of the esterified 1,3-benzylideneglycerols with *N*/40 hydrochloric acid at 80°C., observed a rearrangement to the corresponding α -isomers. King and associates (28, 89), in studies of the effects of acid and base catalysis, observed that β -monopalmitin shifted completely to the α -isomer on standing for 24 hr. in an alcoholic solution of *N*/20 hydrochloric acid or *N*/10 ammonia, and that the β -*p*-bromobenzoate of glycerol shifted to the α -isomer under similar conditions when catalyzed by *N*/10 hydrochloric acid or *N*/10 ammonia. Their observations demonstrated a distinctly greater stability for aromatic monoglycerides and for unsymmetrical diglycerides of the 1,2 type, in agreement with the observations underlying Fischer's early work.

A method suggested by Verkade and associates (94) for the preparation of aliphatic β -monoglycerides was accomplished experimentally by Daubert (17). It involved the catalytic detritylation of the esterified 1,3-ditrityl ether of glycerol, and the reactions were as follows:



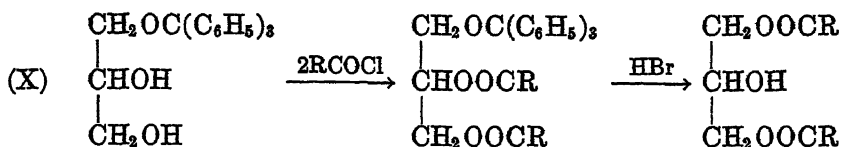
As mentioned previously, acid hydrolysis for the removal of the trityl groups could not be used for the preparation of aliphatic β -monoglycerides because of acyl migration. The above reduction method (IX) and that of Bergmann and Carter (VIII) provide two very satisfactory methods for preparing β -monoglycerides, because good yields of the intermediates can be obtained as highly purified products, and the final steps also give good yields of pure products.

V. PREPARATION OF UNSYMMETRICAL DIGLYCERIDES

In the preparation of diglycerides, the unsymmetrical aliphatic type has offered the most serious difficulty. Grün (48) reported the preparation of symmetrical dimyristin by heating glycerol disulfate with myristic acid dissolved in sulfuric acid, and the unsymmetrical dimyristin by heating the disulfate of α -monochlorohydrin with the fatty acid and subsequently removing the chlorine with silver nitrate. Fairbourne, using Grün's methods, succeeded only in the isolation of the symmetrical diglyceride.

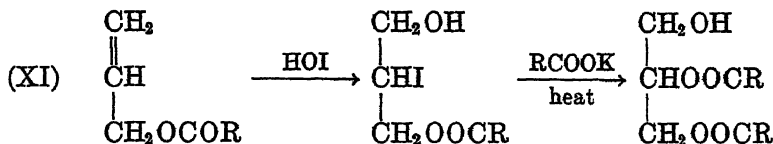
Other investigations (49, 90) have also indicated the unstable character of beta aliphatic groups under such conditions of reaction. Guth (52), and later Renshaw (85), prepared an ester which they thought to be 1,2-distearin, but each probably obtained the symmetrical isomer or a mixture. Heiduschka and Schuster (54) reported the preparation of unsymmetrical dipalmitin and distearin by the methods of Guth, and supposedly proved the configuration of these esters by reaction with thionyl chloride. Fairbourn, however, found that their assumption that thionyl chloride reacts only with primary hydroxyl groups was without foundation, and further, that the unsymmetrical diglycerides isolated by them were probably the symmetrical diglycerides or a mixture of the two isomers. Whitby (100) had apparently prepared 2-palmityl-1,3-distearin from 2-palmityl-1,3-dichlorohydrin by heating the latter with two moles of silver stearate. Thomsen (91) later reported the preparation of 1,2- and 1,3-dimargarins by a method similar to Whitby's, but Fairbourn proved that both isomers melted at the same temperature when purified. Delaby and Dubois (30) reported the preparation of the 1,2- and 1,3-diformates of glycerol by direct esterification, but this method, as well as the methods of Humnicki and Lunkiewicz (73) and Weismann and Haskellburg (99) (for the preparation of diglycerides) apparently involved unrecognized shifting of acyl groups. The preparation of diglycerides has also been reported by Abderhalden and Eichwald (2) and Bergmann (10, 11), using indirect methods with propylamines. Although they obtained evidence of the presence of an unsymmetrical isomer by rotation studies, their products apparently were not pure. While many of the methods (48, 49, 54, 85, 91, 100), including those of Grün and Slepnick (51), in which fatty acid soaps reacted with symmetrical dichlorohydrins and 1-chloro-3-acylglycerol, have been used and may be satisfactory for the preparation of symmetrical diglycerides, they are of no value for the preparation of the unsymmetrical isomers. In several instances it appears that impure halohydrins were used.

A widely used method for preparing symmetrical diglycerides is that of Fischer, Bergmann, and Bärwind (40), using α -iodohydrin (alival), as outlined previously (IV). The investigations of trityl ether reactions (75, 96) have provided an excellent method for the preparation of symmetrical aliphatic diglycerides. Since alival is no longer readily available, and its use involved difficulty in purifying the reaction products, the trityl intermediates may well replace the use of alival. After hydrolysis of the 1-trityl-2,3-diacylglycerols with hydrogen bromide in chloroform, the symmetrical, rather than the unsymmetrical, diglycerides were isolated regularly and with good yields of pure products (X):



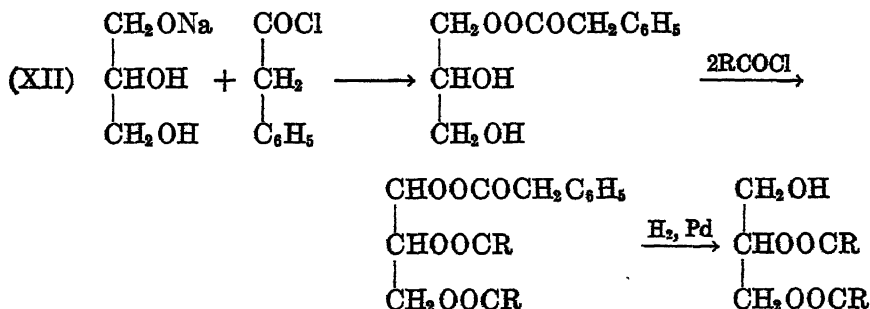
In contrast, Helferich and Sieber (55, 56) were successful in the preparation of aromatic unsymmetrical diglycerides by hydrolytic detritylation of the esterified alpha monotrityl ethers of glycerol.

Golendeev (45) reported the preparation of unsymmetrical diglycerides from allyl esters according to the following reactions:



Although this method has not been fully investigated, it is very improbable that the reactions could be completed without a partial or complete beta-to-alpha shift.

The catalytic detritylation of a 1,2-diacyl-3-tritylglycerol by hydrogen gas in neutral solution, suggested in a paper by Verkade and associates (93), was used successfully by Daubert and King (29). It is apparently the first satisfactory method reported for the preparation of unsymmetrical aliphatic diglycerides. Daubert and King have also reported a new and satisfactory method of preparing 1,2-diglycerides of fatty acids, involving catalytic reduction of esterified 1-glyceryl benzyl carbonate (XII) (29). The latter was prepared with good yields from alpha monosodium glyceroxide and benzyl chloroformate. The resulting 1,2-diglycerides were identical with those prepared by reduction of the esterified alpha trityl ethers. The reactions were as follows:



Because of the unrecognized tendency of acyl groups to migrate and the use of intermediates of uncertain constitution, the methods of Guth and many other investigators (46, 48, 50, 52, 79, 87) for the preparation of

mixed triglycerides containing two fatty acids led to the publication of a large amount of conflicting data for many of the synthetic glycerides. Although the preparation of triglycerides from unsymmetrical diglycerides of definite configuration has been accomplished, the method is obviously hazardous (29) because of the risk of acyl migration. Both β -monoglycerides and symmetrical diglycerides are available for the synthesis and verification of structure of symmetrical mixed triglycerides.

VI. RECENT PHYSICOCHEMICAL STUDIES OF ISOMERIC GLYCERIDES

Malkin and associates (21, 23, 32) have made a detailed thermal and x-ray pattern study of many synthetic mixed triglycerides as well as of the α -monoglycerides and 1,3-diglycerides used as intermediates. They have found that differences in melting and transition points and x-ray data serve to distinguish clearly between the isomeric pairs (*cf.* Ferguson and Lutton⁴).

Clarke and Stegeman (25) have made careful measurements of the specific heats and heats of combustion of the isomeric α - and β -monopalmitins. Their data provide good thermodynamic evidence of the greater stability of the α -forms: "The heats of combustion of alpha- and beta-monopalmitin reduced to the standard state, $-\Delta U_R$, are reported as 2778.78 ± 0.36 and 2788.30 ± 0.67 kcal. per mole for the reaction carried out at one atmosphere pressure and 25° . The difference in the heats of formation or the heat of the shift of the beta form to the alpha form of -9.52 kcal. per mole is in agreement with the chemical behavior of the isomers but is larger in magnitude than had been expected." Judging from their known resemblance in properties, it is likely that isomeric symmetrical and unsymmetrical diglycerides would show similar differences in energy values, but the data for such pairs are not yet available.

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PREPARATION AND PROPERTIES OF OPTICALLY ACTIVE DERIVATIVES OF GLYCEROL¹

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I. INTRODUCTION

Asymmetric substitution in the glycerol molecule produces compounds which are capable of occurring in enantiomorphic forms. This is the case with all α -monoglycerides and α,β -diglycerides as well as α,α' -diglycerides containing unlike substituents and triglycerides in which the α - and α' -positions are substituted by different acyl residues.

This type of asymmetry has been observed in nature in only a few instances. The optically active α -glycerophosphoric acid and the lecithins and cephalins related to it have long been known, while the optically active glycerol ethers such as batyl alcohol have only recently been isolated from natural sources. It might be predicted, however, that the occurrence of glycerides in nature as enantiomorphs is much more widespread than experimental evidence up to the present time indicates. This may be explained by the observation that the optical rotation of stereoisomeric

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triglycerides containing higher fatty acid residues is not detectable. Since the bulk of natural fats is composed of triglycerides, and since nature strongly favors the elaboration of mixed acid and not "simple" triglycerides, it might be expected that this fact has considerable significance. It is conceivable, for instance, that enzymes (e.g., lipase) in their action on the enantiomorphic glycerides might distinguish between the two isomers in an analogous manner to the distinction shown in the case of sugars and amino acids.

The possibility of isolating a pure, optically active individual glyceride from a natural source seems at present remote, since the methods generally employed are such that asymmetry, if present, might be easily destroyed during the process of isolation (acyl migration). The alternative approach to these interesting compounds is to find a reliable means for their synthesis.

A number of methods based on two entirely different principles are available up to the present for the synthesis of optically active glycerides. The first of these general principles requires resolution of intermediate compounds, which then can be converted into the corresponding glycerides. This naturally involves the general difficulty of eliminating, without racemization, the acid or basic residues originally introduced to make possible the resolution. The alternative possibility is to start the synthesis with an optically active natural compound which can be converted without racemization to the desired glycerides. The former principle, involving resolution, has received considerable attention in the laboratories of Abderhalden, Bergmann, and Grün; the second principle has been utilized only recently (7, 8, 9, 10).

Abderhalden and Eichwald (1) resolved racemic aminodibromopropane by means of *d*-tartaric acid. The optical antipodes so obtained were employed to prepare optically active α -monoglycerides and α,β -diglycerides (2), as shown in chart I.

Abderhalden and Eichwald assigned the prefix *d*- or *l*- to these compounds, according to the direction of the observed rotation.

Bergmann and Sabetay (13) resolved the α -acyl esters of γ -aminopropylene glycol by means of saccharic acid, and the resulting enantiomorphs were then treated with nitrous acid to produce α -monoglycerides. Similarly, Bergmann, Brand, and Dreyer (14) converted α,β -diacyl esters of γ -aminopropylene glycol into α,β -diglycerides.

In contrast to the method of Abderhalden and Bergmann, both of whom introduced the basic amino group into the glycerol molecule, Grün and Limpächer (27) attempted the resolution of the glycerides by fractional crystallization of the strychnine salts of their sulfuric acid esters. The potassium salts of the resulting sulfuric acid esters of the glycerides showed extremely high rotations in benzene solutions, the rotations being, how-

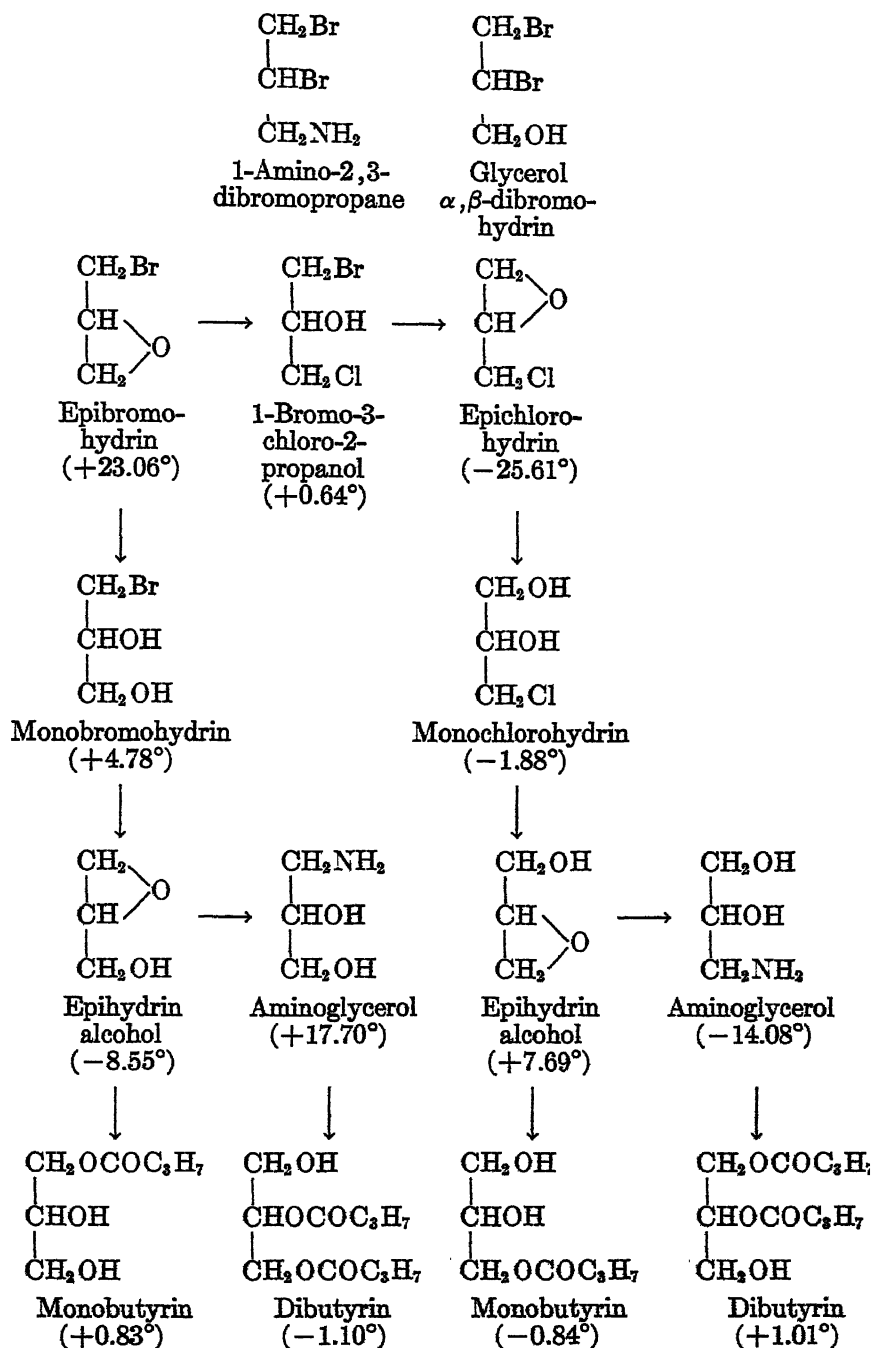
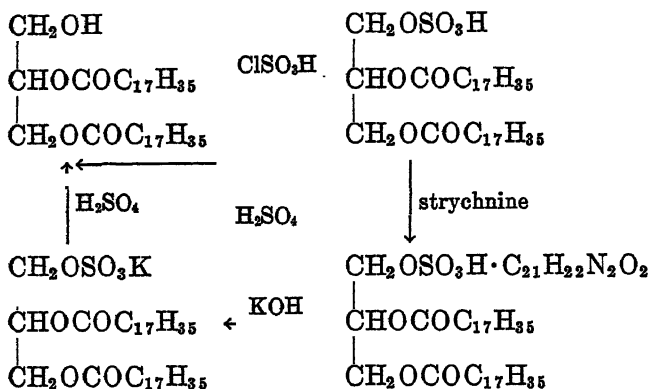


CHART I

ever, dependent on temperature to a surprising degree.² The acid hydrolysis of these sulfuric acid compounds yielded products with no detectable rotation. The reactions as applied to α,β -distearin are represented in the following diagram:



Esters of glycerol with polybasic organic acids are also resolvable as salts of active bases. For example, Suzuki and Inoue (47) resolved the

TABLE 1
Properties of glycerides and intermediates used in the synthesis of glycerides

COMPOUND	OPTICAL ROTATION OF PRODUCT OBTAINED	
	By resolution	From acetoneglycerol
α -Monobutyryn.....	$\pm 0.84^{**}$	$\pm 2.2^\circ$
<i>d</i> (-)- α,β -Distearin.....	$0^\circ \dagger$	-2.7° (in CHCl_3)
α -(<i>p</i> -Nitrobenzoyl)- α',β - dibenzoylglycerol.....	-2.1° (in $\text{C}_2\text{H}_5\text{Cl}_4$) \dagger	-19.9° (in $\text{C}_2\text{H}_5\text{Cl}_4$)
<i>l</i> (+)-Epilhydrin alcohol....	$+7.69^{**}$	$+15^\circ$
γ -Aminopropylene glycol (in HCl solution).....	-14.08^{**}	-29°

* Abderhalden and Eichwald.

\dagger Grün and Limpächer.

\ddagger Bergmann and Sabetay.

half-ester of phthalic acid with α,β -dibenzoylglycerol by means of strychnine into the stereoisomeric forms which were optically active.

The second general principle available for the synthesis of optically active glycerides, as mentioned previously, makes use of optical activity already present in a natural product capable of being converted without

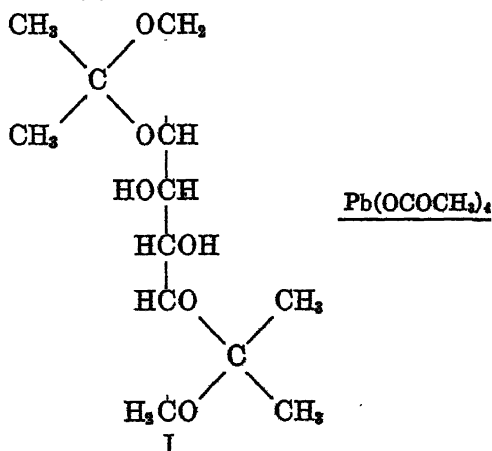
² A similar dependence of rotation on temperature has been observed with certain phosphatides and phosphatidic acids (27, 41, 43).

racemization to the desired glyceride. This might be compared with the transformation of carbohydrates into optically active fats, which nature probably carries out by enzymatic reactions in plants and in animal bodies. This principle has been employed by the authors during the last few years and has led to the preparation of a number of active glycerides. The method involves the use of *d*- and *l*-mannitol, from which, by appropriate reactions, the enantiomorphic *d*(+)- and *l*(-)-acetoneglycerols can be obtained (6, 7). Acylation of these compounds according to the method of Fischer, Bergmann, and Bärwind (24), which will be described later, produced the various enantiomorphic glycerides. In all instances observed, this principle of using an inherent natural activity appeared to be preferable to any method involving the principle of resolution, since the glycerides and intermediates invariably possessed greater optical activity than those in corresponding compounds prepared by resolution. Some data on glycerides and related compounds used as intermediates for glyceride synthesis, prepared by both methods, are compared in table 1.

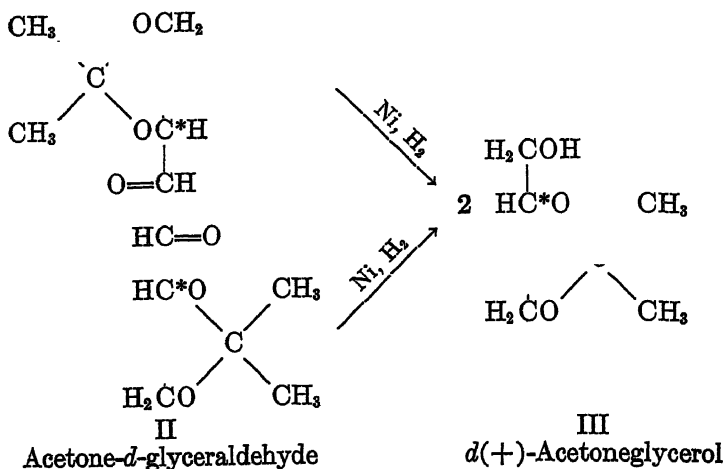
II. PREPARATION OF THE ENANTIOMORPHIC ACETONEGLYCEROLS

1,2,5,6-Diacetone-*d*-mannitol (I) was prepared by the action of acetone and zinc chloride on *d*-mannitol. The carbon chain of the diacetone-*d*-mannitol was split by lead tetraacetate, with the result that two molecules of acetonated *d*-glyceraldehyde (II) (7) were formed. This compound was reduced catalytically in ethyl acetate solution with hydrogen under pressure, using the Raney nickel catalyst or the nickel catalyst according to Rupe (42), and yielded *d*(+)-acetoneglycerol (III) in an excellent yield.

Starting with *l*-mannitol, exactly the same procedure was followed to produce *l*(-)-acetoneglycerol. However, unlike *d*-mannitol, which is a



1,2,5,6-Diacetone-*d*-mannitol

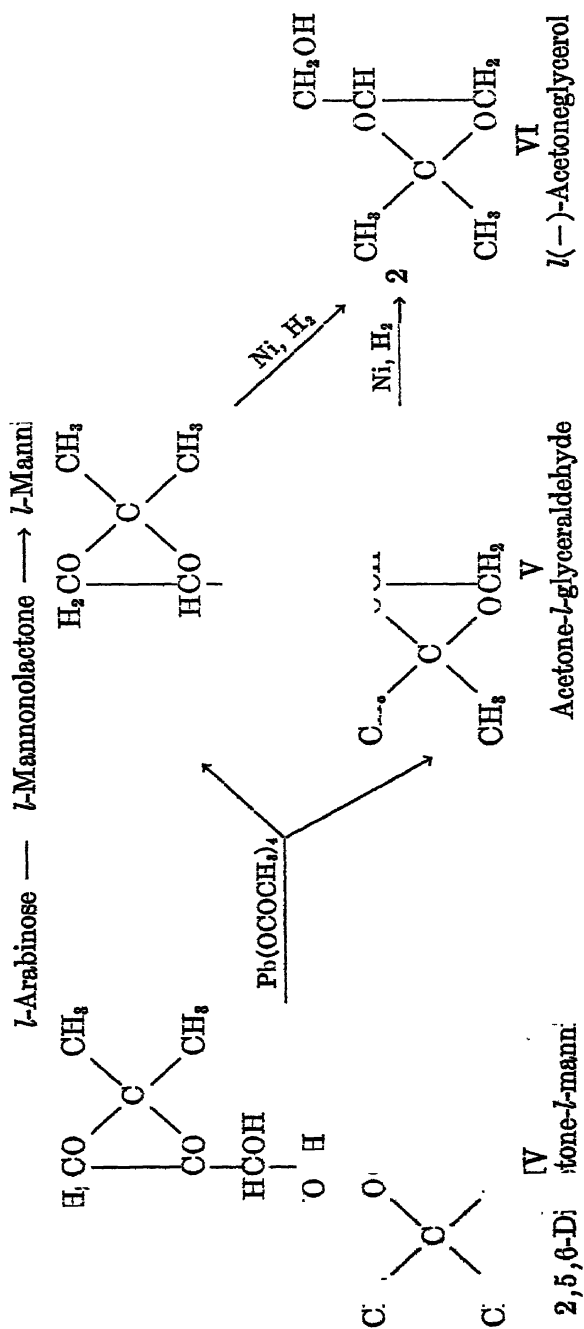


commercially available substance, *l*-mannitol must be prepared by a tedious series of reactions starting from *l*-arabinose. The *l*-arabinose itself is obtained from Arizona mesquite gum by the method of Anderson and Sands (4). The arabinose is then transformed by cyanohydrin synthesis into *l*-mannonolactone (33), and the latter is reduced catalytically in one operation to *l*-mannitol (20). Cleavage of the 1,2,5,6-diacetone-*l*-mannitol (IV) with lead tetraacetate then produces two molecules of acetone-*l*-glyceraldehyde (V). The reduction of this compound with nickel and hydrogen leads to the formation of *l*(-)-acetoneglycerol (VI).

The rotations of the two enantiomorphous acetoneglycerols prepared in this way were found to be of equal magnitude ($[\alpha]_D = \pm 12.6^\circ$). Since the acetoneglycerols were prepared from 1,2,5,6-diacetonemannitols of known structure (15), their constitution as 1,2-acetoneglycerols was also established. As an additional proof of structural uniformity, the *d*(+)-acetoneglycerol was converted by methylation and hydrolysis to glycerol α -monomethyl ether. The latter, on titration with periodic acid (7), proved to be pure glycerol α -methyl ether. The optical rotation of *d*(+)-acetoneglycerol was unaltered by successive benzylation, purification of the high-boiling benzoate, and saponification to the original, optically active acetoneglycerol.

It should be pointed out that the asymmetry of the acetone-*d*-glyceraldehyde is maintained in the glycerol series only by the initial presence of the isopropylidene group in the asymmetrical 1,2-position. Should the molecule become symmetrical at any stage in the operations, a subsequent asymmetrical substitution would yield only a racemic compound.

By an appropriate series of reactions in which the asymmetry is maintained throughout, *d*(+)-acetoneglycerol can be transformed into *l*(-)-acetoneglycerol (25). The reactions shown in chart II are involved.



The interconversion of optical antipodes is reminiscent of the work of Emil Fischer and Brauns (22) on the *d*- and *l*-isopropylmalonic acids, in which the asymmetry was maintained by substituents on the two carboxyl groups.

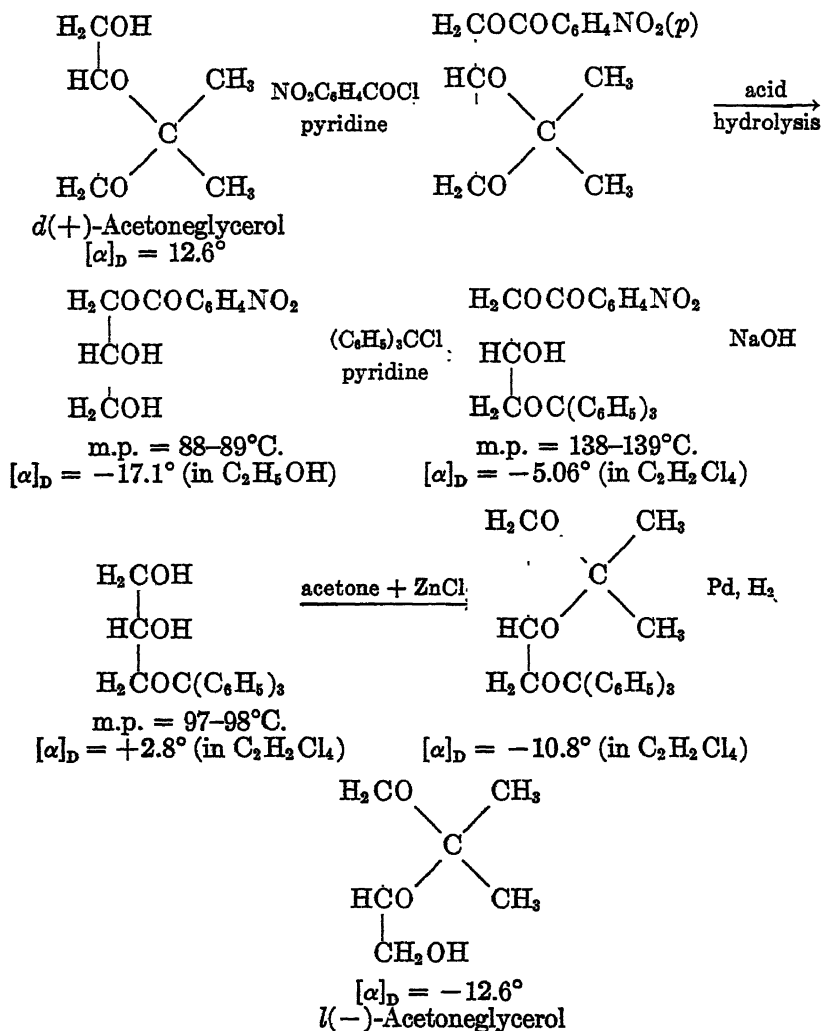


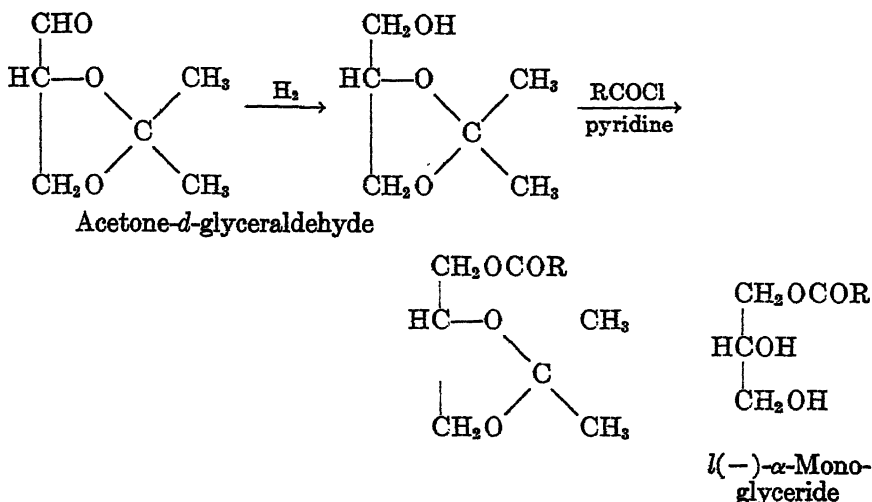
CHART II

III. PREPARATION OF GLYCERIDES

A. α -Monoglycerides and triglycerides

Possession of the *d*(+)- and *l*(-)-acetoneglycerols opened the way for the preparation of optically active glycerides, according to the method of

synthesis used by Emil Fischer, Bergmann, and Bärwind (24) for the corresponding racemic compounds. This method consists in introducing the acyl group into the free hydroxyl position by the action of acid chloride and quinoline or pyridine. Mild acid hydrolysis then removes the protecting acetone group, yielding an optically active α -monoglyceride. The synthesis of an α -monoglyceride from acetone-*d*-glyceraldehyde is represented by the following equations:



The acetonated α -monoglycerides listed in table 2 have been prepared from *d*(+)-acetoneglycerol.³

By acid hydrolysis of the corresponding acetone compounds, the optically active α -monoglycerides listed in table 3 have been obtained.

Since there is a certain danger of racemization, due to acyl migration, during the acid hydrolysis of the protecting acetone group, a number of experiments were conducted to ascertain the purity of the enantiomorphous α -monoglycerides. Titration of the 1,2-glycol content with lead tetraacetate by the methods of Criegee (16) and Malaprade (38) showed the products to be pure α -monoglycerides. This evidence, however, does not eliminate the possibility that racemization may have occurred during the acid treatment. Only if re-acetonation of the monoglyceride results in an α -acyl- α' , β -acetoneglycerol of the correct rotation can it be concluded that the free monoglyceride is a pure enantiomorph.

The ease of removal of the acetone residue by means of acid hydrolysis varies considerably with the nature of the acyl residue in the acetonated

³ Note the change of nomenclature from that used in reference 8. The acetone compounds are now given the same steric classification as the monoglycerides prepared from them.

TABLE 2

Acetonated l- α -monog.

ACETONE COMPOUND OF	BOILING POINT °C.	MELTING POINT °C.	REFRACTIVE INDEX	ROTATION	SPECIFIC ROTATION	SOLVENT
<i>l</i> (-)- α -Acetylglycerol.....	85-86 at 10-11 mm.	°C.	$n_D^{15} = 1.4288$		$[\alpha]_D = +3.24^\circ$	Homogeneous substance
<i>l</i> (-)- α -Propionylglycerol.....	88-89 at 7 mm.		$n_D^{20} = 1.4260$		$[\alpha]_D = +3.6^\circ$	Homogeneous substance
<i>l</i> (-)- α -Butyrylglycerol.....	97-98.5 at 7 mm.		$n_D^{20} = 1.4270$		$[\alpha]_D = +4.92^\circ$	Homogeneous substance
<i>l</i> (-)- α -Caproylglycerol.....	118-120 at 7 mm.		$n_D^{20} = 1.4322$		$[\alpha]_D = +4.5^\circ$	Homogeneous substance
<i>l</i> (-)- α -Laurylglycerol.....	130-131 at 0.002 mm.		$n_D^{20} = 1.4448$		$[\alpha]_D^{20} = +3.42^\circ$	Homogeneous substance
<i>l</i> (-)- α -Stearylglycerol.....		43.5		$\alpha_D^{20} = +3.0$ to $+3.5$		Fused sub- stance, 1 dm. tube
<i>l</i> (-)- α -Palmitylglycerol.....		33-35		$\alpha_D = +4.38^\circ$	$[\alpha]_D = +1.9^\circ$	Pyridine Fused sub- stance, 1 dm. tube
					$[\alpha]_D = +2.48^\circ$	Pyridine

α -monoglyceride, and consequently the conditions of the hydrolysis must be adapted in each instance to the specific compound in hand.

TABLE 3
l- α -Monoglycerides

COMPOUND	MELTING POINT	SPECIFIC ROTATION	SOLVENT
	$^{\circ}\text{C.}$		
<i>l</i> (-)- α -Butyrylglycerol (liquid).....	{	$[\alpha]_{\text{D}} = -6.0^{\circ}$	Pyridine
		$[\alpha]_{\text{D}} = -2.2^{\circ}$	Homogeneous substance
<i>l</i> (-)- α -Laurylglycerol.....	53-54	$[\alpha]_{\text{D}} = -3.76^{\circ}$	Pyridine
<i>l</i> (-)- α -Stearylglycerol.....	76-77	$[\alpha]_{\text{D}} = -3.58^{\circ}$	Pyridine
<i>l</i> (-)- α -Palmitylglycerol.....	71-72	$[\alpha]_{\text{D}} = -4.37^{\circ}$	Pyridine
<i>l</i> (-)- α -(<i>p</i> -Toluenesulfonyl)glycerol.....	63-64	$[\alpha]_{\text{D}} = -7.3^{\circ}$	Pyridine
<i>l</i> (-)- α -(<i>p</i> -Nitrobenzoyl)glycerol.....	88-89	$[\alpha]_{\text{D}} = -17.1^{\circ}$	Ethanol

TABLE 4
Acetone compounds of d- α -monoglycerides; *d*(+)- α -butyrylglycerol

SUBSTANCE	BOILING POINT	REFRACTIVE INDEX	SPECIFIC ROTATION	SOLVENT
	$^{\circ}\text{C.}$			
Acetone compound of: <i>d</i> (+)- α -Propionylglycerol.....	88-89 at 7 mm.	$n_{\text{D}}^{24} = 1.4269$	$[\alpha]_{\text{D}} = -3.6^{\circ}$	Homogeneous substance
<i>d</i> (+)- α -Butyrylglycerol.....	97-99 at 7 mm.	$n_{\text{D}}^{23} = 1.4280$	$[\alpha]_{\text{D}} = -4.9^{\circ}$	Homogeneous substance
<i>d</i> (+)- α -Caproylglycerol.....	118-120 at 7 mm.	$n_{\text{D}}^{20} = 1.4349$	$[\alpha]_{\text{D}} = -4.5^{\circ}$	Homogeneous substance
Free α -monoglyceride: <i>d</i> (+)- α -Butyrylglycerol.....			$[\alpha]_{\text{D}} = +2.2^{\circ}$	Homogeneous substance

From the difficultly available *l*(-)-acetoneglycerol were prepared the three acetone compounds of α -monoglycerides and one free α -monoglyceride listed in table 4.]

In the active α -monoglycerides containing aromatic substituents, the rotations remain unchanged after standing for a period of one year, indicating that the acyl residues do not undergo migration. This is in agreement with the findings of Jackson and King (29), who observed no acyl migration in glycerides containing aromatic acid radicals. The α -monoglycerides derived from aliphatic acids, however, show a gradual decrease of rotation even in the crystalline state at room temperature, amounting to one-half to one-third of the original rotation during one year (9).

In view of this instability of the pure monoglycerides *in vitro*, one is led to speculate on the possibility of enantiomorphic glycerides existing and retaining their steric configuration *in vivo*. The recent work of Schoenheimer (44) has brought forth new evidence that the fats involved in metabolism in the animal body undergo rapid and constant chemical change. Any such lability in the case of the glycerides taking part in fat metabolism would appear at first sight to make impossible the existence of any stable stereoisomerism. However, it is conceivable that the interchange of acyl residues of the glycerides *in vivo* would not necessarily result in racemization, but rather that the configuration of the glycerol part of the molecule would be retained during these chemical changes by the specific action of the enzymes, and that this configuration would comprise, indeed, one of the stable characteristics of the cell.

From the α -monoglycerides, triglycerides containing different acyl residues can be obtained by further treatment with acid chlorides and quinoline or pyridine. To eliminate the possibility that the original materials—the active α -monoglycerides—had become racemized by migration of the acyl group under the influence of the tertiary base (pyridine or quinoline) used in the above preparations, the monoglycerides were exposed for some time to the action of pyridine. There was, however, no change in rotation observable, and the glycerides, when recovered from the solvent, were shown by titration by the method of Criegee to be pure α -monoglycerides. From *l*(-)- α -lauryl-, *l*(-)- α -stearyl-, *l*(-)- α -palmityl-, and *l*(-)- α -(*p*-nitrobenzoyl)-glycerol, respectively, the triglycerides listed in table 5 were prepared.

In spite of the distinct rotation shown by the monoglycerides, most triglycerides prepared from them show no rotation whatever in the solvents used, either in sodium light or in ultraviolet light. This possession of asymmetry without measurable optical activity may well be a characteristic of triglycerides with fatty acid residues, because α -(*p*-nitrobenzoyl)- α , β -dibenzoylglycerol possesses the considerable rotation of $[\alpha]_D = -19.9^\circ$.

The lack of observable rotation in the synthetic aliphatic unsymmetrical triglycerides with at least two different long-chain fatty acid residues leads to the suspicion that the *natural* triglycerides of the same type may also

show no rotation. Therefore, natural asymmetric triglycerides, though they do not show a rotation, are not necessarily racemic, but might easily occur in either of the two possible enantiomorphic forms.

Since, in contrast to the triglycerides, the α -monoglycerides show a relatively large rotation, it is possible that the slight and gradually disappearing rotation sometimes observed (46, 47) in freshly isolated natural fats and oils is due to the presence of α -monoglycerides, especially since, as mentioned before, synthetic α -monoglycerides show a gradual decrease in rotatory power on standing at room temperature.

B. Steric classification and nomenclature of glycerides

With regard to the question of steric relationship, the optical classification of the enantiomorphic α -monoglycerides is established comprehensively by relating them to the corresponding *d*- and *l*-glyceraldehydes.

TABLE 5
Synthetic triglycerides prepared from α -monoglycerides

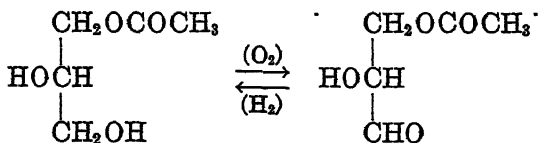
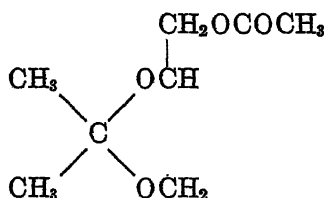
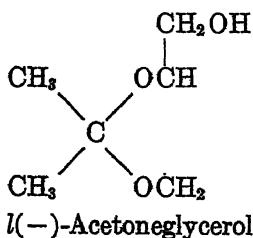
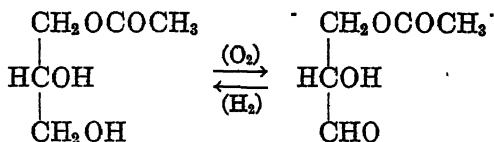
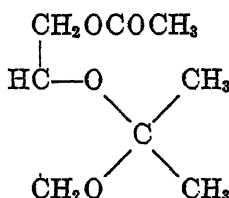
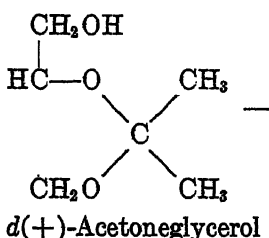
TRIGLYCERIDE	MELTING POINT	α_D
	°C.	
α -Lauryl- α' , β -distearyllycerol.....	48.5	0.0° (in pyridine)
α -Stearyl- α' , β -dipalmityllycerol.....	62.5	0.0° (in chloroform or pyridine)
α -Palmityl- α' , β -dilauryllycerol.....	44	0.0° (in pyridine)
α -(<i>p</i> -Nitrobenzoyl)- α' , β -distearyllycerol..	67-67.5	-1.4° (in chloroform)
α -(<i>p</i> -Nitrobenzoyl)- α' , β -dibenzoyllycerol..	87-88	-19.9° (in tetrachloro- ethane)

This concept has a precedent in the classification of the sugars according to Wohl and Freudenberg (52). Thus an α -monoglyceride which would be formed by the reduction of a 3-acyl-*d*-glyceraldehyde is called a *d*- α -monoglyceride, while a glyceride originating from a 3-acyl-*l*-glyceraldehyde is an *l*- α -monoglyceride. In other words, an α -monoglyceride is to be put in the same category with that glyceraldehyde into which it could be transformed by oxidation without any alteration or removal of substituents. This opinion is directly opposed to that of Abderhalden and Eichwald (1), who hold the view that *no* steric classification of the α -monoglycerides is possible, since members of either series may be obtained from the same original material.

In the preparation of the α -monoglycerides from acetone-*d*-glyceraldehyde through *d*(+)-acetoneglycerol by acylation of the newly formed hydroxyl group and splitting off the acetone, the new substituent has been introduced into the hydroxyl group of that carbon atom which was part

of the carbonyl group of the original acetone-*d*-glyceraldehyde. The α -monoglycerides prepared from *d*(+)-acetoneglycerol therefore belong to the *l*-glyceraldehyde series and are to be designated *l*-glycerides, for if one were to oxidize the α -monoglycerides thus obtained, the result, according to the assumptions discussed above, would be 3-acyl-*l*-glyceraldehydes. Therefore a change of configuration has taken place, and we find ourselves faced with the remarkable fact that application of the sequence of reactions mentioned above to a derivative of *d*-glyceraldehyde yields a derivative of *l*-glyceraldehyde. This transformation from one steric series into the other one is made possible only by the peculiar kind of asymmetry in the glycerol series, an asymmetry which depends entirely on *substitution*.

The way from *d*(+)-acetoneglycerol to an acyl derivative of *l*-glyceraldehyde, and from *l*(-)-acetoneglycerol to a derivative of *d*-glyceraldehyde, is demonstrated below:



This reasoning can be extended without constraint to the α,β -diglycerides, the α -glycerophosphoric acids, and the α -glycerol ethers. In these

classes of compounds the α -position occupied by the free hydroxyl group is considered to be the potential carbonyl group of that glyceraldehyde to which the particular compound is to be related.

In the case of mixed acid triglycerides this steric classification is arbitrary but may be applied if a chemical or biological consideration gives preferred importance to one of the α -substituents. To illustrate this viewpoint the following example may be cited:

Consider an α -phosphoryl α',β -distearin arising from *l*(-)-glycerophosphoric acid by the introduction of two stearyl residues. If this triglyceride is to be subjected to phosphatase action, its relation to the original *l*(-)-glycerophosphoric acid would govern the course of the hydrolysis, and consequently the relationship to *l*-glyceraldehyde should be maintained and expressed in the nomenclature. On the other hand, if the same triglyceride is to be subjected to the action of lipase, the stearyl groups are primarily affected, and the compound would be classified as a derivative of *d*- α,β -distearin and hence related to *d*-glyceraldehyde.

C. α,β -Diglycerides⁴

Optically active *d*(+)-acetoneglycerol has also been used to prepare what is believed to be the first example of an optically pure enantiomorph of an α,β -diglyceride, namely *d*(-)- α,β -distearin. Attempts to prepare this type of optically active glyceride, as previously indicated in the introduction, have been based on the principle of resolution of racemic intermediate compounds. Abderhalden and Eichwald (2) prepared the enantiomorphie epihydrin alcohols through resolution, and converted these to the corresponding enantiomorphie α -aminopropylene glycols. The amino alcohols were then converted to optically active α,β -diglycerides by the introduction of two *O*-acyl groups, followed by elimination of the amino group with nitrous acid. In a somewhat similar way, Bergmann, Brand, and Dreyer (14) resolved the di-*O*-benzoate of α -aminopropylene glycol. Elimination of the amino group with nitrous acid produced an α,β -dibenzoin which was converted to an optically impure *p*-nitrobenzoate.

By a series of reactions starting with *d*(+)-acetoneglycerol, it has recently been demonstrated⁴ that the epihydrin alcohols and α -aminopropylene glycols used by Abderhalden and Eichwald were not optically pure,

⁴ The section on α,β -diglycerides was written by John C. Sowden and is based on experimental work carried out by him in this laboratory. The publications of John C. Sowden and H. O. L. Fischer pertaining to the α,β -diglycerides and epihydrin alcohol will appear in the near future, as communications X and XI in the series of "Studies on Acetoneglyceraldehyde and Optically Active Glycerides" by H. O. L. Fischer and Erich Baer.

and thus the enantiomorphic diglycerides described by them must also have been optically impure.

The reactions involved in the preparation (and proof of optical purity) of *l*(+)-epihydrin alcohol⁵ and *l*(-)- α -aminopropylene glycol⁵ are represented in the scheme in chart III.

It appears safe to assume that the steps involved in scheme A are such that no racemization can occur. Thus the reactions in scheme B must also have proceeded without racemization, and the *l*(+)-epihydrin alcohol

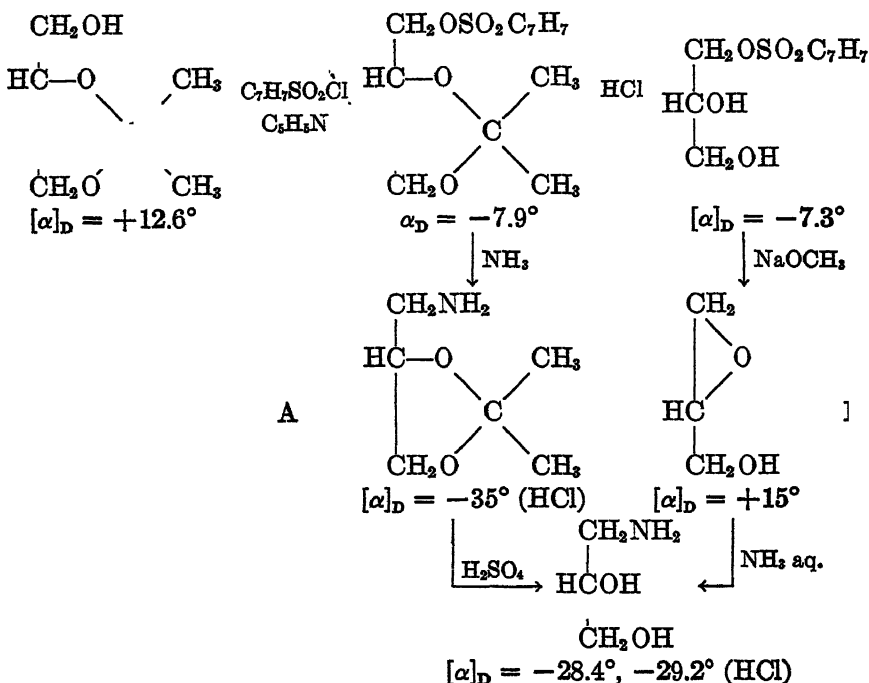


CHART III

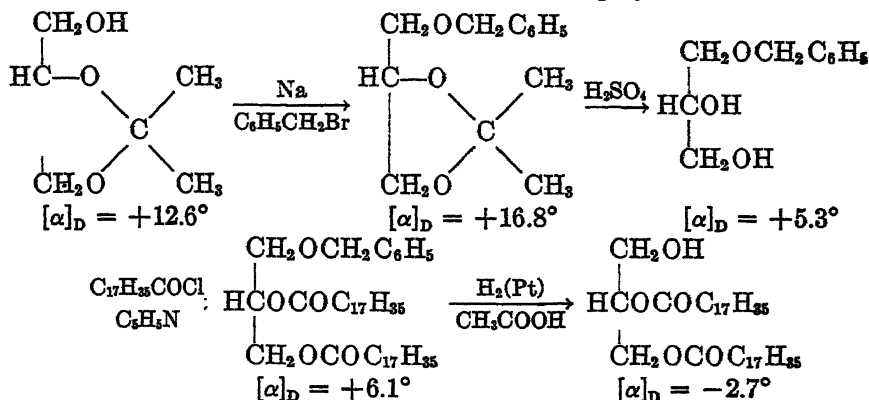
and *l*(-)- γ -aminopropylene glycol are optically pure. Apparently no measurable amount of 1,3-oxide is produced in the formation of the epihydrin alcohol from *l*(-)- α -tosylglycerol, since this isomeric oxide would produce racemic γ -aminopropylene glycol in the next step, and result in a lowering of the optical activity.

In agreement with the observation of L. Knorr and E. Knorr (36), who worked with racemic epihydrin alcohol, the addition of ammonia to *l*(+)-epihydrin alcohol apparently yields exclusively the α -amine. Moreover,

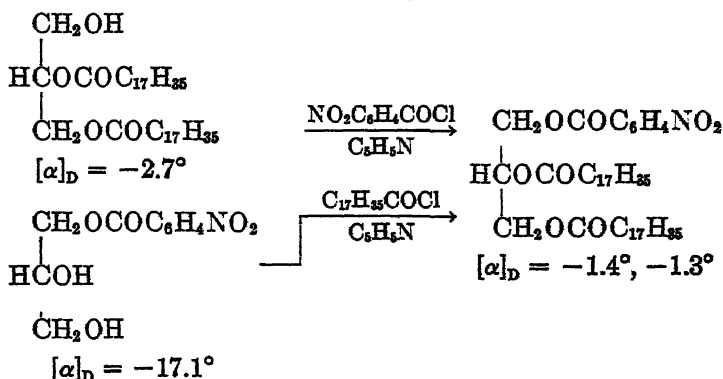
⁵ The prefix "*l*" is assigned here by considering the epihydrin alcohol as related to the oxide of *l*-glyceraldehyde and the γ -aminopropylene glycol as related to an *l*-glyceraldehyde in which the hydroxyl on carbon atom 3 is replaced by the amino group.

the addition seems to proceed without Walden inversion, since the resulting amine has the same configuration as that obtained from α -tosyl- $d(+)$ -acetoneglycerol. This latter observation is interesting in the light of the results of Levene and Walti (37), who observed that the degree of Walden inversion which occurs during the addition of water to optically active propylene glycol depends largely on the pH of the solution.

For the preparation of optically pure $d(-)$ - α,β -distearin from $d(+)$ -acetoneglycerol, the following reactions were employed:



As proof of the optical purity of the diglyceride, it was converted to the p -nitrobenzoate and compared with the product from the known $l(-)$ - α -(p -nitrobenzoyl)glycerol (25) and stearyl chloride.



It is a necessary condition that the α,β -diglyceride containing aliphatic ester groups shall at no time be subjected to the effect of mineral acids, since these have been demonstrated to cause acyl migration (21, 29). The benzyl ether was chosen for the diglyceride synthesis because it is stable to acid and alkali but can be removed readily by hydrogenation.

The benzyl group was introduced by the reaction of benzyl bromide with the sodium salt of $d(+)$ -acetoneglycerol, the latter being prepared

either with metallic sodium in anhydrous ethyl ether or with sodium naphthalene (45, 51) in dimethyl glycol ether. In order to demonstrate that the reaction with sodium caused no racemization in the acetoneglycerol molecule, the sodium salt was also used through reaction with methyl iodide, to prepare the known methyl ether of *d*(+)-acetoneglycerol, $[\alpha]_D = +22.5^\circ$ (7).

In the removal of the benzyl group from the distearin benzyl ether with platonic oxide (Adams catalyst) and hydrogen, it was found that no hydrogenation occurred when ethyl acetate was used as solvent. However, hydrogenation proceeded smoothly in glacial acetic acid at room temperature. Acetic acid in the cold apparently caused no migration of the acyl groups, as evidenced by the proof of asymmetry through the distearin *p*-nitrobenzoate.

The optically active distearin was also converted to the acetate, but the resulting triglyceride showed no perceptible optical rotation. This is in agreement with the observation of Baer and Fischer (8) that asymmetric triglycerides prepared up to the present time, containing only aliphatic acid residues, show no detectable optical rotation.

IV. PREPARATION OF GLYCEROPHOSPHATES

It has been demonstrated (8) by the synthesis of optically active α -monoglycerides that asymmetrical α -monoglycerides of both plant and animal origin should also show activity when present only in one of the enantiomorphic forms. A causal relationship between optical activity and the asymmetry of the glycerol molecule in naturally occurring glycerides has not yet been established with complete validity. The chief reason for this appears to be the difficulty of isolating pure substances from natural fats, which consist of nearly inseparable mixtures of many glycerides, without altering the original constitution and configuration in the process. Suzuki and Inoue (47) have found that freshly extracted natural fats are optically active and show a declining rotation. Unequivocal proof that this activity results from the asymmetry of the glycerol molecule in natural glycerides has not yet been obtained.

In the case of α -glycerophosphoric acid, which has long been known as a component of the lecithins and cephalins and may in a sense be looked on as a monoglyceride, the situation is much more favorable. Because of its great stability several workers have been able to isolate it from various phosphatides and free it from its accompanying β -isomer (12, 26, 31).

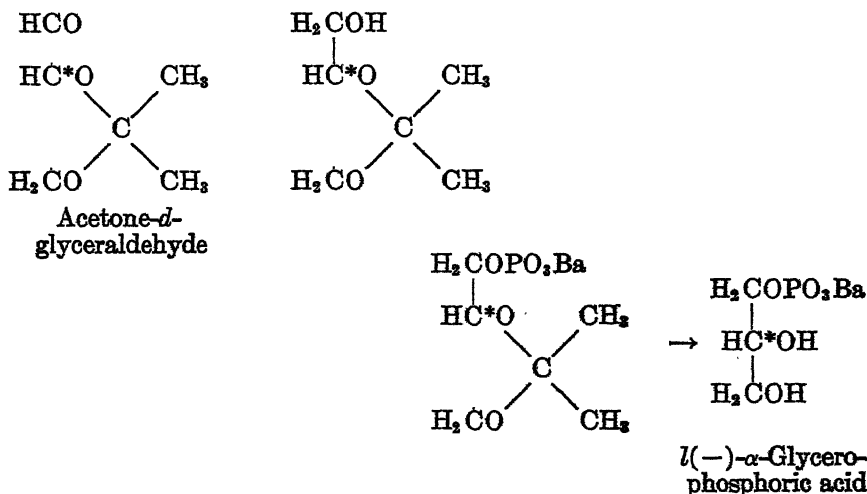
By preparing the comparatively highly active dimethyl ether dimethyl ester of α -glycerophosphoric acid from its barium salt, which shows no perceptible rotation, Karrer and Salomon (31) were able to show conclusively that the α -glycerophosphoric acid in the phosphatides examined

by them is optically active and present in only one enantiomorphic form. Meyerhof and Kiessling (39) later established its identity with the glycerophosphoric acid which forms an intermediate stage in alcoholic fermentation and glycolysis, and observed that the biological *l*(-)- α -glycerophosphoric acid is completely utilized by the enzyme of muscle press juice, while its antipode remains entirely unaltered.

The preparation of the enantiomorphic forms of α -glycerophosphoric acid was undertaken by Abderhalden and Eichwald (3) and by Karrer and Benz (30). However, Abderhalden and Eichwald obtained a non-homogeneous product, while Karrer and Benz were successful only in partially resolving the synthetic α -glycerophosphoric acid of Emil Fischer and Pfähler (23) through the quinine salt, as they showed by comparison of the rotation of the dimethyl ether dimethyl ester of their product with the corresponding derivative of the natural glycerophosphoric acid.

Moreover, the steric relationship between the α -glycerophosphoric acids and compounds of known configuration (active glyceraldehydes, for example) could not be deduced from any information then available. The preparation of the two active α -glycerophosphoric acids was therefore undertaken by the authors, starting from *d*(+)- or *l*(-)-acetoneglycerol, for the purpose of relating their configuration in the sense of Wohl and Freudenberg to that of the active glyceraldehydes.

The sequence of reactions involved was the same as that employed by Emil Fischer and Pfähler (23) for the synthesis of racemic α -glycerophosphoric acid. The enantiomorphic acetoneglycerols were phosphorylated by means of phosphorus oxychloride and quinoline. The protective acetone groups were removed and the α -glycerophosphoric acids isolated as their barium salts. The following equation illustrates the preparation of *l*(-)- α -glycerophosphoric acid from acetone-*d*-glyceraldehyde:



The steric classification of the two glycerophosphoric acids is established along the same lines of reasoning as discussed above for the aliphatic α -monoglycerides. Thus $d(+)$ -acetoneglycerol gives rise to the $l(-)$ - α -glycerophosphoric acid, while $l(-)$ -acetoneglycerol yields $d(+)$ - α -glycerophosphoric acid.

The identity of the synthetic $l(-)$ - α -glycerophosphoric acid with the "biological α -glycerophosphoric acid" from glycolysis, alcoholic fermentation, and from phosphatides was established by comparing the rotation of the dimethyl ether dimethyl esters:

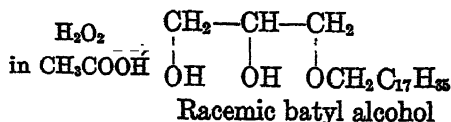
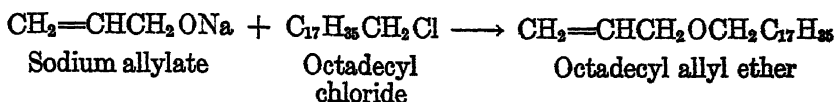
- (a) Synthetic..... $[\alpha]_D = -4.78^\circ$
- (b) From phosphatides..... $[\alpha]_D = -3.2^\circ$ (31)
- (c) From glycolysis and fermentation..... $[\alpha]_D^{20} = -4.46^\circ$ (39)

In agreement with the work of Karrer and Salomon, the barium salt of the synthetic $l(-)$ - α -glycerophosphoric acid showed no perceptible rotation. The weak rotations given in the literature for the barium salt of α -glycerophosphoric acid from phosphatides may therefore be ascribed to the presence of optically active impurities. Further, the silver salt of the synthetic acid shows a specific rotation of only $[\alpha]_D = +1.0^\circ$ (in ammoniacal solution). Kiessling and Schuster (32) found $[\alpha]_D = +0.8^\circ$ (saturated aqueous solution) for the silver salt of the natural α -glycerophosphoric acid from fermentation. The diethyl ester of the diethyl ether ($[\alpha]_D^{20} = -5.31^\circ$, in homogeneous substance) may in future investigations prove to be a valuable preparation for comparison. A rotation of $[\alpha]_D^{20} = +5.94^\circ$ has been observed for the corresponding derivative of $d(+)$ - α -glycerophosphoric acid (11).

V. α -GLYCEROL ETHERS

The occurrence of monoethers of glycerol with higher fatty alcohols, such as octadecyl, cetyl, and oleyl alcohols, in the liver oils of various marine fish, especially those of the Elasmobranch group, was first demonstrated by Tsujimoto and Toyama (50), and later by Nakamiya (40). André and Bloch (5) showed that in the original fish oils these ethers are present in the form of fatty acid esters. From 1928 to 1933 a thorough investigation into the structure of inactive batyl and chimyl alcohols was carried out by Heilbron and Owens (28), Davies, Heilbron, and Owens (17), and Davies, Heilbron, and Jones (18).

Their synthesis (17) of racemic batyl alcohol was accomplished by the condensation of octadecyl chloride with sodium allylate, and subsequent oxidation of the octadecyl allyl ether with perhydrol.



The racemic α -cetyl glycerol ether was prepared in the same way, using cetyl chloride for condensation. These syntheses and the lead tetraacetate titration of the ethers (16) proved that batyl alcohol, chimyl alcohol, and selachyl alcohol must be the α -glycerol ethers of octadecyl alcohol, hexadecyl alcohol, and oleyl alcohol, respectively.

Since substitution of glycerol in the α -position causes the β -carbon atom to become asymmetric, Davies, Heilbron, and Jones (18) were led to examine again the optical properties of natural batyl alcohol. They reported that the natural alcohol has a slight optical activity, $[\alpha]_D^{20} = +2.6^\circ$ (in chloroform, $c = 0.95$), an observation in disagreement with an earlier finding of Toyama (48).

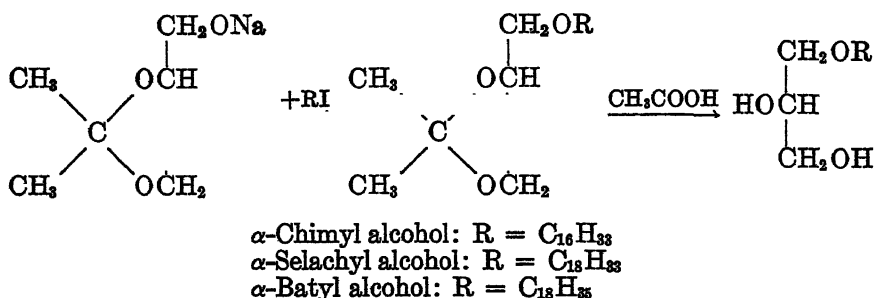
Recently, Toyama and Ishikawa (49) made the observation that the rotations of free batyl and selachyl alcohols in chloroform and ethyl alcohol are largely dependent on the concentrations used. For instance, while the selachyl alcohol in substance showed a specific rotation of $[\alpha]_D^{26} = -4.33^\circ$, increasing dilution with chloroform or ethyl alcohol resulted in a gradual decrease in value, until at concentrations of approximately 10 per cent no rotation was detectable. When dilutions below 10 per cent were employed a dextrorotation was observed. Similarly, batyl alcohol at a concentration of 3.15 g. per 100 cc. in chloroform solution showed $[\alpha]_D = +2.14^\circ$, while at a concentration of 9.94 g. per 100 cc. in ethyl alcohol the rotation was approximately zero. These observations of Toyama and Ishikawa reconcile the disagreement of Toyama's earlier observation with that of Davies, Heilbron, and Jones (18).

In this laboratory it was found that the two enantiomorphic forms of the synthetic, like the natural, batyl alcohol in a concentration of 10 per cent in chloroform showed no detectable rotation, but their diacetates showed rotations in good agreement with that recorded for the diacetate from natural batyl alcohol (18).

The constitutions of the three natural α -glycerol ethers and the fact that they occurred in one enantiomorphic form were thus well established, although the steric classification of these compounds, or their relationship to glyceraldehyde, was still to be determined. With this object in view, the syntheses of both enantiomorphic forms of the optically active α -glyc-

erol ethers were undertaken, and carried out in such a way that in all stages of the process the optical relations between the resulting ethers and the *d*- and *l*-glyceraldehydes, the reference compounds, could be clearly traced.

The *d*(+)-acetoneglycerol and *l*(-)-acetoneglycerol, which had proved so useful in the synthesis of optically active mono-, di-, and tri-glycerides and glycerophosphates, were again used as starting materials. For the present synthesis, the sodium salts of the acetoneglycerols were brought into reaction with hexadecyl and octadecyl iodides in boiling glycol dimethyl ether (45, 51), yielding the acetone compounds of α -hexadecyl and α -octadecyl glycerols. Hydrolysis with acetic acid gave the free alcohols, which are identical with chimyl alcohol (m.p. 62–63°C.) and batyl alcohol (m.p. 71°C.), respectively, the configuration of which is represented below:

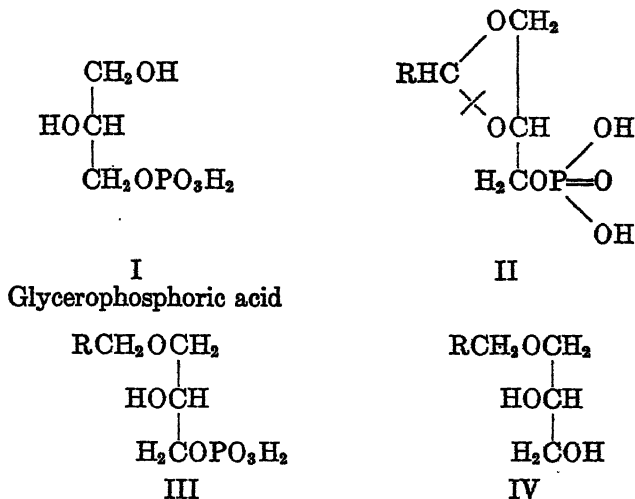


The diacetylated synthetic batyl alcohols showed a specific rotation of $[\alpha]_{5461}^{20} = \pm 8.6^\circ$ (in chloroform, $c = 11.2$). Davies, Heilbron, and Jones had found $[\alpha]_{5461}^{20} = -8.5^\circ$ (in chloroform, $c = 2.63$) for the natural compound (18). For comparison of the rotation of the acetone compound of natural batyl alcohol with that of the corresponding synthetic compound, a crude preparation of the glycerol ethers from the unsaponifiable fraction of ratfish (*Chimaera Monstrosa*) liver oil (m.p. 61.5–62.5°C., containing mainly chimyl alcohol) was acetonated. An acetone compound was obtained with the specific rotation of $[\alpha]_D = -14.0^\circ$ (in substance). The two acetone compounds of the synthetic chimyl and batyl alcohols had specific rotations of $[\alpha]_D = \pm 12.1^\circ$ and $[\alpha]_D^{40} = \pm 12.6^\circ$, respectively (in melted substance). Since the rotations of derivatives of the natural batyl alcohol agree with those of the derivatives of α -octadecylglycerol synthesized from *l*(-)-acetoneglycerol, it must be concluded that the batyl alcohol belongs to the *d*-series (see above). The steric relationship was assigned according to the principles used for the classification of the α -monoglycerides and glycerophosphates (page 299 of this article). Selachyl alcohol also belongs to the *d*-series, because it can be transformed by catalytic reduction to *d*-batyl alcohol (50).

Polarimetric examination of synthetic chimyl alcohol in chloroform at

a concentration of 10 per cent showed no perceptible rotation, in agreement with the finding of Toyama and Ishikawa (49). However, the acetone compound and the diacetyl derivative of the synthetic product prepared from *l*(-)-acetoneglycerol showed a rotation of the same sign as that of natural chimyl alcohol. Therefore natural chimyl alcohol belongs to the *d*-series, for the same reason that batyl alcohol does.

It is conceivable that these ethers (IV) are formed in the cell by reductive splitting of the acetal phosphatides (plasmals) (19), II \rightarrow III,



and subsequent removal of phosphoric acid, III \rightarrow IV. The fact that the natural glycerophosphoric acid (I) belongs to the *l*-series, and that the glycerol ethers, as far as they have been investigated, belong to the *d*-series, is in agreement with the above hypothesis.

In other words, it appears that etherification and esterification take place in nature in the α - and α' -positions, respectively, which in asymmetrically substituted glycerols are no longer equivalent.

It is also interesting to point out that the glycerol acetals with fatty acid aldehydes might be related to the corresponding fatty acid glycerides by an oxidation and reduction mechanism. Thus it would not be surprising if future investigation reveals that these glycerol acetals play an important rôle in fat metabolism.

VI. BIOCHEMICAL CHARACTERISTICS OF THE ENANTIOMORPHS

In most cases it would be difficult to follow, in intact animals, the metabolism of enantiomorphic compounds of the types considered here, but it was felt that some indication of the behavior of these enantiomorphs in the animal body might be obtained by studying the action of enzymes

on these compounds in isolated systems. Investigations to establish the significance of optical asymmetry were therefore begun, using various glycerol derivatives, such as glycerophosphates, mono-, di-, and tri-glycerides and glycerol ethers and acetals with fatty acid residues.

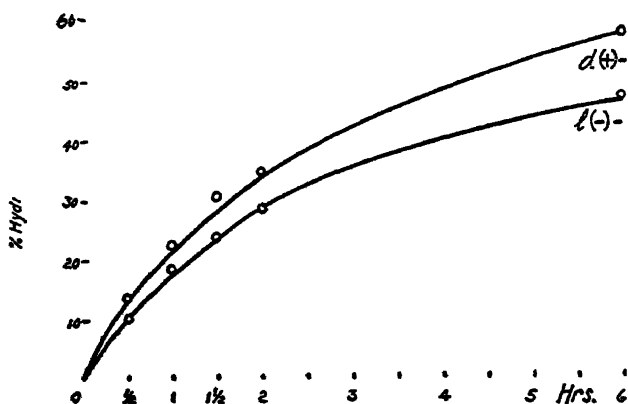


FIG. 1. Hydrolysis of *l*(-)- and *d*(+)- α -glycerophosphates by pig kidney phosphatase. 8 cc. of ammonium buffer (pH, 9.6), 8 cc. of substrate (containing 8.0 mg. of organic phosphorus), and 4 cc. of enzyme solution were used.

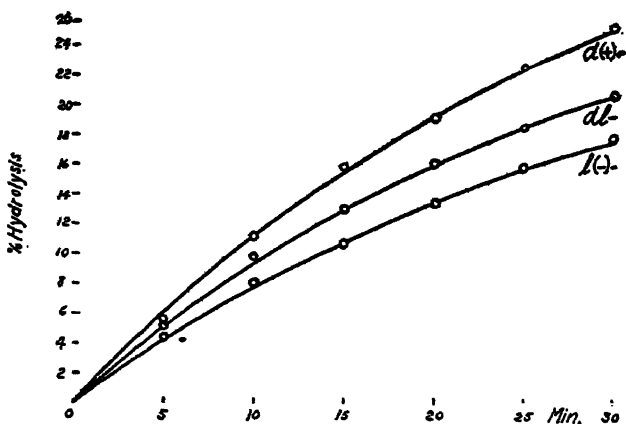


FIG. 2. Hydrolysis of inactive, *l*(-)-, and *d*(+)- α -glycerophosphates by phosphatase prepared from dog feces. 25 cc. of carbonate-veronal buffer (pH, 8.62), 20 cc. of substrate (containing 6.2 mg. of organic phosphorus), and 5 cc. of enzyme solution were used.

A. Phosphatases

A study of the action of phosphatases on glycerophosphates was undertaken first, because the glycerophosphates are less liable to acyl migration

than glycerides with fatty acid residues, and because it was already known that the two enantiomorphic α -glycerophosphates react differently with the ferments of muscle press juice. Meyerhof and Kiessling (39) found, using the racemic compound as substrate, that only the $l(-)$ - α -glycerophosphoric acid was used up completely, the $d(+)$ - α -glycerophosphoric acid remaining unchanged.

Experiments repeated by Meyerhof⁶ with samples of the synthetic pure $l(-)$ - and $d(+)$ - α -glycerophosphoric acids have confirmed the previous result.

The rate of hydrolysis of $l(-)$ - α -glycerophosphoric acid and $d(+)$ - α -glycerophosphoric acid by unrefined kidney phosphatase, taka-phosphatase, rat bone phosphatase, and a purified phosphatase from dog feces has now been investigated (11). It was shown with all four preparations that, under the conditions specified, the $d(+)$ - α -glycerophosphoric acid was hydrolyzed with greater velocity than the $l(-)$ - α -glycerophosphoric acid. This fact has probably some physiological significance.

Typical results obtained with two enzymes—namely, kidney phosphatase with ammonium buffer (pH, 9.6) and phosphatase from dog feces with carbonate-veronal buffer (pH 8.62)—are illustrated by the curves shown in figures 1 and 2. The method used to follow the rate of hydrolysis was the same as that described by King and Armstrong (35), and the determination of free phosphoric acid was carried out according to King (34).

The racemic compound is hydrolyzed, as was to be expected, at a rate approximately midway between those of its two components. Taka-phosphatase with phthalate buffer (pH, 3.8) and rat bone enzyme with carbonate-veronal buffer (pH, 8.62) gave essentially similar results.

B. Lipases⁷

In contrast to the α -glycerophosphates, which show no tendency to migrate, the glycerides containing fatty acid residues are known to be quite susceptible to migration. For this reason two kinds of derivatives of α -monoglycerides, in which migration of the acyl residue in alkaline media was made impossible by substituting the β - and α' -hydroxyl groups, were chosen to study the action of lipases.

The investigation was first carried out with the acetone compounds of stereoisomeric α -butyryl- and α -caproylglycerols. The results are given in figure 3.

It is apparent from the curves that, under the experimental conditions

⁶ Private communication.

⁷ Unpublished results of Erich Baer and H. O. L. Fischer.

used, the acetone compounds of the *d*-series (prepared from *l*(-)-acetone-glycerol) were hydrolyzed by the lipase of the guinea pig serum with approximately twice the velocity observed for the corresponding antipodes. The velocity of hydrolysis of the racemic compounds is just intermediate between the velocities observed for the pure isomers, thus substantiating these latter values.

Analogous experiments were carried out with the dimethyl ethers of the enantiomorphous α -butyrylglycerols as substrates. The blood serum and liver extract of guinea pigs, rabbits, and rats were used as a source of lipase. Under the conditions used (0.04 molar substrate solution; pH,

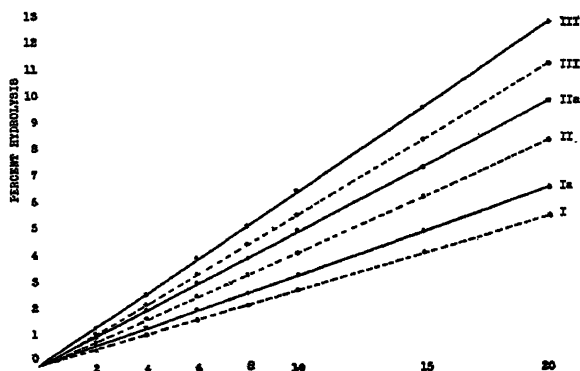


FIG. 3. Hydrolysis of the acetone compounds of the enantiomorphous α -butyryl- and α -caproyl-glycerols. Curve I, acetone compound of *l*(-)- α -*n*-butyrylglycerol; curve II, acetone compound of inactive α -*n*-butyrylglycerol; curve III, acetone compound of *d*(+)- α -*n*-butyrylglycerol; curve Ia, acetone compound of *l*(-)- α -*n*-caproylglycerol; curve IIa, acetone compound of inactive α -*n*-caproylglycerol; curve IIIa, acetone compound of *d*(+)- α -*n*-caproylglycerol. The hydrolysis was carried out by the "continuous titration" technique at pH 7.2 in a mixture containing 10 cc. of 0.1 *M* substrate solution (in gum acacia), 10 cc. of water, 1 cc. of phenol red indicator, and 0.4 cc. of guinea pig serum.

7.2; temperature, 37°C.; "continuous titration"), it was found that one enantiomorph, in this case the dimethyl ether of the *l*(-)- α -butyrylglycerol, was hydrolyzed with a velocity 1.2 to 3.5 times higher than the other.

These two series of experiments, using acetonated and methylated monoglycerides as substrates, clearly demonstrate that the asymmetry of the β -carbon atom has an influence on the action of enzymes on glycerides.

The free enantiomorphous monoglycerides are, in many instances, difficult to prepare in an optically pure state, and are easily racemized. However,

investigation has shown that the free enantiomorphous α -butyrins are hydrolyzed with different velocities by the lipase of guinea pig serum. The observed difference is largely dependent on concentrations of the substrates, as is shown by the following example: In 0.3 molar substrate solution one α -monobutyrim was hydrolyzed with a velocity 14 per cent greater than its antipode, while in 0.05 molar substrate solution a difference in velocity of hydrolysis of 85 per cent was observed.

Further investigations regarding enzyme action on synthetic enantiomorphous glycerol derivatives are being carried out in this laboratory.

VII. BIOLOGICAL CONCLUSIONS TO BE DRAWN FROM THE CONFIGURATION OF THE NATURAL α -GLYCEROPHOSPHORIC ACID

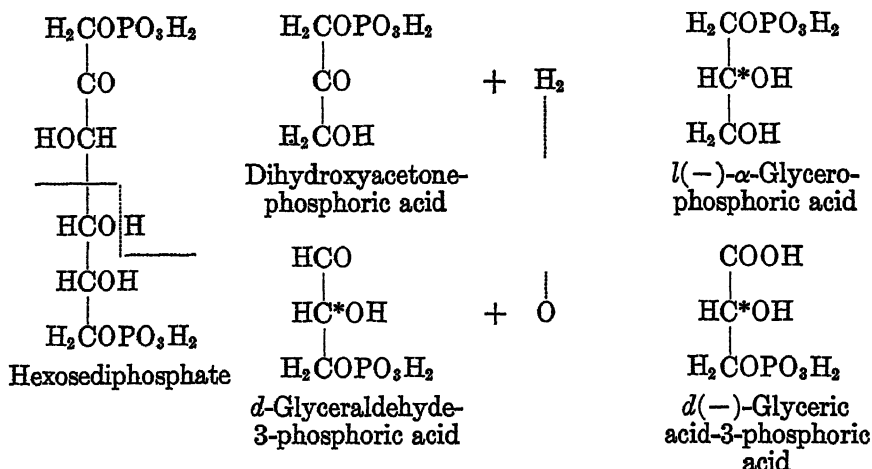
Once the steric relationship of the α -glycerophosphoric acids to the glyceraldehydes has been fixed by synthesis from the two enantiomorphous acetoneglycerols, certain biological conclusions may be drawn.

In the fermentation scheme of Embden, as developed by Meyerhof, Kiessling, and Lohmann, the dismutation of triosephosphoric acids plays an important part. Since α -glycerophosphoric acid is the product of simple reduction of triosephosphoric acids, it follows from our purely chemical evidence that the natural $l(-)$ - α -glycerophosphoric acid does not arise biologically from the natural d -glyceraldehyde-3-phosphoric acid, because the latter belongs to another steric series, but that it must have been formed by asymmetrical fermentative reduction from dihydroxyacetonephosphoric acid.

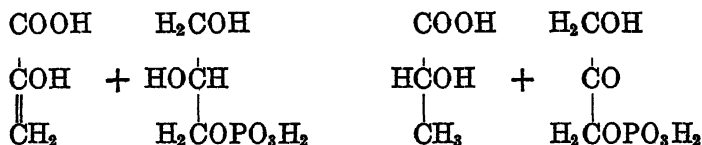
The steric configuration of $l(-)$ - α -glycerophosphoric acid having been established by synthesis (10), it became a comparatively simple matter to determine the steric configuration and consequently the steric relationship between the biological α -glycerophosphoric acid from yeast and the natural levorotatory glyceric acid-3-phosphoric acid.

Kiessling and Schuster (32) oxidized the biological $l(-)$ - α -glycerophosphoric acid with bromine and obtained, as might be predicted from the relationship already established by synthesis, not the natural glyceric acid-3-phosphoric acid but its antipode $l(+)$ -glyceric acid-3-phosphoric acid, which has no counterpart in nature.

This is further evidence that the two dismutation products of triosephosphoric acids, α -glycerophosphoric acid and glyceric acid-3-phosphoric acid, belong to two different steric series. Thus the natural glyceric acid-3-phosphoric acid must belong to the d -series and accordingly can arise by oxidation only from d -glyceraldehyde-3-phosphoric acid. Summing up these results, we obtain the accompanying scheme for the dismutation of triosephosphoric acids:



In the light of this new steric relationship the well-known Embden-Meyerhof reaction of pyruvic acid and α -glycerophosphoric acid, which has hitherto been assumed to yield lactic acid and *d*-glyceraldehyde-3-phosphoric acid, must now be written thus:



The fact that *l*-glyceraldehydephosphoric acid is not utilized biologically (as has been demonstrated from experience with *d*,*l*-glyceraldehyde-3-phosphoric acid) compels the conclusion that dihydroxyacetonephosphoric acid must be formed in this reaction.

Both enantiomorphic α -glycerophosphoric acids are ideal starting materials for the synthesis of phosphatide acids and phosphatides of which the configuration is known with regard to the β -carbon atom of the glycerol residue. Since only the *l*(-)- α -glycerophosphoric acid has so far been found in natural substances, it appears likely that most of the α -phosphatides which have as yet been investigated are derived from this acid, rather than from its antipode, and consequently the configuration of the glycerol part of these phosphatides is established.

It is probable that an important rôle is played by *l*(-)- α -glycerophosphoric acid in the transformation of carbohydrate into fat in the bodies of higher animals. It seems to be the substance which, formed from phosphorylated sugars, in fermentation and glycolysis, would supply the asymmetry for the synthesis of optically active α -phosphatides and fats in nature.

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THE SEPARATION OF NATURAL COMPONENTS OF FATS AND OILS BY MOLECULAR DISTILLATION^{1,2}

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I. INTRODUCTION

"Molecular distillation" is the term used for the process of distilling material from a heated layer to a nearby condenser across space so highly evacuated that the mean free path of the distilling molecules is equal to or greater than the distance from the evaporating surface to the condensing surface. In practice, the distillation is usually done on thin layers of material at a pressure of about 1 micron (0.001 mm.) of mercury, and the condenser is placed about 1 cm. away from the distilling surface. When a small amount (less than 20 ml.) of material is to be distilled, a "pot" still is used (figure 1). A shallow puddle of distilland on a heated surface is located immediately below a condensing surface.

When a larger amount of material is to be distilled, a "falling-film" still is used (figure 2). The distilland flows in a thin film over a heated surface located near a condensing surface. The design most popular on both a laboratory and a commercial scale uses two vertical concentric cylinders.

¹ Presented at the Symposium on the Molecular Structure of Fats and Oils, which was held under the auspices of the Division of Biological Chemistry and the Division of Agricultural and Food Chemistry at the 101st Meeting of the American Chemical Society, St. Louis, Missouri, April 7-11, 1941.

² Communication No. 22 from the Laboratories of Distillation Products, Inc.

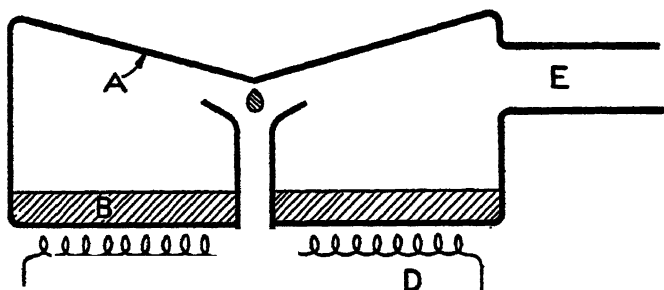


FIG. 1. Simplified diagram of a pot still. A, condensing surface; B, distilland; C, distillate; D, heating element; E, connection to vacuum pumps.

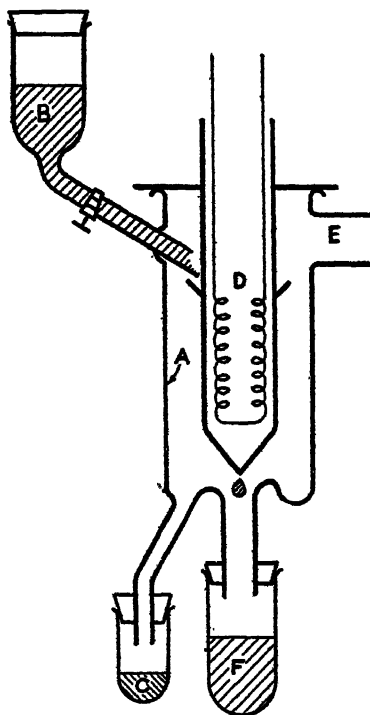


FIG. 2. Simplified diagram of a falling-film still. A, condensing surface; B, distilland; C, distillate; D, heating element; E, connection to vacuum pumps; F, undistilled residue.

The distilland flows down over the outside of the inner cylinder, and the distillate collects on and flows down the inner surface of the outer cylinder.

Readers interested in all the modifications of molecular stills described in the scientific and patent literature should refer to the bibliographies on molecular distillation compiled by Detwiler and Markley (10) and Detwiler (9), which give over two hundred references. There will appear soon in this journal a complete review (30) of the history of molecular distillation and of its scientific accomplishments. This present article is limited in scope to a discussion of the distillations that have been carried out on natural animal and vegetable oils, except for a brief mention of analytical distillations.

II. AMOUNT OF SEPARATION TO BE EXPECTED

Since in molecular distillation practically all of the molecules of vapor leaving the distilling surface are caught on the condenser, there is no "fractionation" in the usual sense of the word. Burch (6) and Washburn (45) have pointed out that the rate of molecular distillation of substances may be calculated by the Langmuir (36) equation:

$$n = PA \sqrt{\frac{2\pi \bar{M} RT}{M}}$$

when n = rate in moles per second,

p = vapor pressure in dynes per square centimeter,

A = area of distilling surface in square centimeters,

M = molecular weight,

R = ideal gas constant, and

T = temperature in °K.

Assuming a perfect solution of two substances, the relative enrichment of the condensate will be $(P_1/M_1) \div (P_2/M_2)$ instead of $P_1 \div P_2$, the factor for the simplest ordinary distillation. The separations will be the same as that in an ordinary distillation if $M_1 = M_2$ and may be better or worse if $M_1 \neq M_2$, depending on the magnitude of the relative enrichment ratio. The first example of the case where molecular distillation had a better degree of fractionation than ordinary distillation was reported by Brønsted and Hevesy (5). They distilled mercury containing two isotopes in a molecular pot still and found that the isotopes which had the same vapor pressure were partially separated, owing to their different molecular weights. Although substances present with other materials of similar vapor pressures and molecular weights cannot be purified with a reasonable number of distillations, it is often possible to study their distillation characteristics and even to identify them by means of the analytical distillations that are discussed in the next section.

III. STEPWISE OR ANALYTICAL DISTILLATIONS

The study of the distillation of the more volatile constituents, such as the free fatty acids or vitamins, from an animal or vegetable oil has been carried out with the use of a "cyclic" molecular still. The cyclic still is a small falling-film still equipped with two oil reservoirs and an oil-circulating pump so arranged that the oil being distilled may be passed over the evaporating surface of the still as many times as desired without requiring any interruptions, such as breaking the vacuum to recharge the still or to remove the undistilled residue. It is also equipped with a means to remove the distillate samples without breaking the vacuum.

"Analytical" distillations are carried out on the cyclic still in the following manner: The still is charged with a solvent oil that is substantially non-volatile in the temperature range to be investigated (the least volatile fraction of corn oil is often used), and dissolved in the solvent oil is a small amount of the substance to be investigated. After the vacuum has been established, the oil solution is passed over the evaporating surface at room temperature a few times to remove the traces of dissolved air and water. The temperature of the evaporation surface is then raised to a point where the substance under study just begins to distill. After all the oil has passed over the evaporating surface at this temperature, the distillate is removed, the oil is passed over the evaporating surface at a slightly higher temperature, and the distillate again is removed. This cycle of operations is repeated until ten to twenty distillate samples are obtained corresponding to a series of temperatures, which, as a rule, are separated by constant intervals of 5 or 10 degrees.

In practice, there is added to the oil solution a small amount of mixed triglycerides made from C_4 to C_{12} fatty acids (2). A fraction of this "constant yield oil" distills at each temperature to wash the distillate into the distillate receiver. Furthermore, impurities (often up to 99 per cent) in the preparation of the substance under examination act as constant yield oil and do not invalidate the distillation unless they affect the determination of the relative concentration of the substance in the distillates. The distillation requires the presence of only enough of the substance so that it may be assayed in the five or six most potent distillates.

The amounts of the substance in the distillates increase for the first few temperatures, because the vapor pressure of the substance increases with each increase in the temperature. But when the amount of the substance left in the distilland is nearly exhausted, the amounts in the distillate will become less and less until the substance is finally completely distilled. The progress of the distillation is best shown by an "elimination curve," the curve drawn through the points obtained by plotting the relative yield

of the substance in the distillates against the temperature of the distillation (figure 3). Under controlled conditions of distillation the temperature corresponding to the maximum of the elimination curve was found, by Hickman (25) on experimental grounds and by Embree (11) on theoretical grounds, to be as characteristic of the substance as a boiling point.

The temperatures of the elimination maxima and the shape of the elimination curves have been used to characterize the nature of various substances present in fish-liver oils that could not be separated in their pure states. Analytical distillations have shown (26) that vitamins A and D occur in fish-liver oils both in the free (alcohol) state and esterified with fatty acids. The shapes of the elimination curves for the vitamin

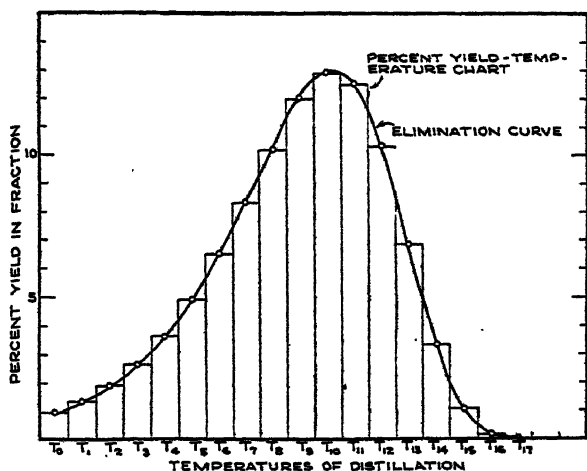


FIG. 3. Elimination curve (Embree (11))

esters show that several different fatty acids are present in the vitamin esters.

The elimination curves for a series of fatty acids were determined by Gray and Cawley (23). The curves they obtained for lauric, myristic, palmitic, and stearic acids are shown in figure 4, and those for the C_{18} acids,—stearic, oleic, linoleic, 9,11-linoleic, and α -eleostearic,—in figure 5. The overlapping of the elimination curves shows the poor separation that could be expected by a single molecular distillation of a mixture of these acids. Therefore, molecular distillation is not as suited as the conventional vacuum distillation methods for the separation and analysis of mixtures of fatty acids.

Molecular distillation will probably contribute a great deal to the study of certain specialized problems on fatty acids. A notable instance was the work done on the acids of cod-liver oil by Farmer and Van den

Heuvel (14). It had previously been thought by Webb (50) and Toyama and Tsuchiya (43) that the principal acid was "clupanodonic," a pentaene C_{22} acid. When the cod-liver oil acids were separated by molecular distillation, no clupanodonic acid was found, and the principal acid was a hexaene C_{22} acid. Ordinary methods of distillation had changed the natural hexaene acid into the artifactual clupanodonic acid.

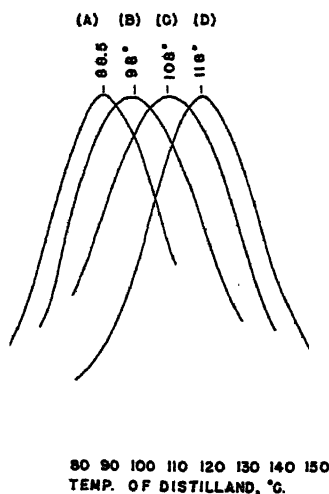


FIG. 4

FIG. 4. Elimination curves of four saturated fatty acids. A, lauric acid; B, myristic acid; C, palmitic acid; D, stearic acid. (Gray and Cawley (23)).

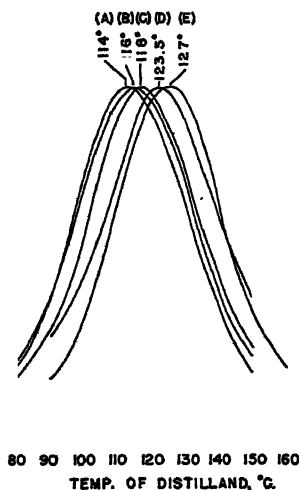


FIG. 5

FIG. 5. Elimination curves of four unsaturated fatty acids and stearic acid. A, linoleic acid; B, oleic acid; C, stearic acid; D, 9,11-linoleic acid; E, α -eleostearic acid. (Gray and Cawley (23)).

IV. DISTILLATIONS OF ANIMAL AND VEGETABLE OILS

Oils (including fats) when expressed or extracted from animal and vegetable tissue usually contain the following types of materials: (1) phospholipids and mucilaginous matter; (2) free fatty acids; (3) substances with potent flavors and odors; (4) unsaponifiable matter, including sterols, vitamins, antioxidants, and other alcohols, ethers, and hydrocarbons; (5) triglycerides.

Molecular distillation cannot be carried out with oils that contain more than traces of phospholipids and mucilaginous matter. These materials cause excessive foaming and the deposition of carbonaceous matter on the distilling surface. The substances responsible for this trouble, however, may be satisfactorily removed by refining processes involving treatment with acid, alkali, or salt solutions (15, 44).

A. Distillation of free fatty acids from oils

Tables 1 to 6 give some of the properties of the fractions from the molecular distillation of various oils. The free fatty acids are concentrated in the first fractions in all the distillations.

B. Distillation of materials with strong flavors and odors from oils

The odoriferous and flavoring materials distill into the first fractions when the oil is molecularly distilled (27), and may therefore be removed from the bulk of the oil.

When the flavoring ingredients are valuable instead of a nuisance, removing them by molecular distillation will enable them to be recovered without the loss by hydrolysis, pyrolysis, or aqueous extraction which is inherent in the conventional steam distillation process. For example: Waterman and van Vlodrop (49), by molecular distillation, removed the first fraction from butter. This fraction, amounting to about 5 or 10 per cent of the original butter, contained all of the flavoring matter and could be used to flavor margarine or other food products.

C. Distillation of unsaponifiable matter from oils

The distillation data of various oils shown in tables 1 to 6 show that the unsaponifiable matter is concentrated in the first few fractions. The exceptions to this general rule that have been met with so far occur with oils such as dogfish-liver oil where the unsaponifiable material is partly composed of the glyceryl ethers known as batyl, selachyl, and chimyl alcohols. These diacidic alcohols occur naturally as the di-fatty acid esters, and these esters have substantially the same vapor pressures and distillation characteristics as the triglycerides (35).

Hydrocarbons. Baxter (1) found hydrocarbons with probable formulas ranging from $C_{15}H_{32}$ to $C_{19}H_{40}$ occurring in small amounts in the very first fraction of distillate from a commercial distillation of cod-liver oil. Hickman (29) found considerable quantities of sesquiterpenes and hydrocarbons from the commercial distillation of mixed vegetable oils. It seems likely that the small fractions from large commercial batches of oils will yield hydrocarbons in quantities large enough for complete characterization.

Sterols. The unsaponifiable materials from most oils are mainly sterols. Hickman and Tischer (33) have described general processes for obtaining sterols from natural oils, and Fawcett and Myles (17) have described a process for concentrating stigmasterol from soybean oil. Both processes are based on the fact that the sterols distill before the main bulk of the triglycerides. Hickman (29) points out that there is little chance of sepa-

rating the sterols from one another by a single molecular distillation because their elimination maxima are so close together.

Antioxidants (tocopherols). It was noticed by Hickman and Baxter (32) and Fawcett (16) that early fractions from the distillation of vegetable oils were considerably more stable towards oxidation than the later fractions and the original oil, and that these early fractions would confer increased stability on other oils when admixed with them. Riemen-schneider *et al.* (41) confirmed this in a study of cottonseed oil. Tocopherol concentrates are now being prepared in commercial quantities by molecular distillation (29).

Vitamins. In addition to the concentration of tocopherols (vitamin E), molecular distillation has been used to prepare concentrates of other fat-soluble vitamins by the distillation of natural oils.

Vitamin A. It has been known for some time that vitamin A concentrates could be made by the molecular distillation of fish-liver oils (7, 20, 24). Vitamin A was found by Hickman (26) to be concentrated in two separate fractions during a single distillation. Experiments showed that the lower boiling fraction contained vitamin A in the alcohol form and the higher boiling fraction contained fatty acid esters of vitamin A. The bulk of the vitamin A occurs in the ester form, and, since the vitamin A esters are more stable to oxidation (42) and are generally more active biologically (13, 21, 22, 37), the preparation of vitamin A concentrates by molecular distillation on a commercial scale was both feasible and desirable. In the United States, Distillation Products, Inc., are now producing distilled vitamin A ester concentrates on a large scale. In England, the British Drug Houses, Ltd., have been distilling vitamin A alcohol from the unsaponifiable fraction of fish-liver oils on a commercial scale since 1934.

The large-scale distillations of fish-liver oils have made possible the isolation of minute quantities of other substances related to vitamin A. Of these, "cyclized" vitamin A (12), which distills before and with the vitamin A alcohol, has no biological activity, but interferes with both the ultraviolet absorption method and the antimony trichloride blue-color method of assaying vitamin A.

Carotene. Waterman and van Vlodrop (49) distilled red palm oil completely and found that the carotene was not concentrated in any one fraction, but that all the distillates had substantially the same red color as the original oil. But when the combined distillates were again distilled, the carotene was concentrated in the first fractions, as it is when a solution of carotene in a bleached oil is distilled. No explanation for this effect has been given.

Vitamin D. Vitamin D also may be distilled from fish-liver oils. A small amount of vitamin D alcohol appears before the main fraction of the

fatty acid esters of vitamin D (26). Although the present type of commercial falling-film stills causes a certain amount of destruction of vitamin D by pyrolysis, new designs being developed distill vitamin D from fish-liver oils with a very good recovery (30).

Vitamin K. Vitamin K is concentrated in the first fractions from soybean oil, but less than six human daily doses can be recovered from a gallon of the crude oil (31). The availability of cheap synthetic anti-hemorrhagic agents has rendered such a concentrate of little commercial value.

D. Separation of the triglycerides

It has been shown above that molecular distillation gives very little separation for mixtures of fatty acids. Since natural oils are "mixtures

TABLE 1
The molecular distillation of linseed oil
Hickman (1938)

	ORIGINAL	FRACTION 1*	FRACTION 2	FRACTION 3	FRACTION 4	RESIDUE
Iodine No.....	177.9	148.7	148.0	173.3	187.9	190
Saponification No.....	192.5	171.3	163.1	183.3	194.2	184.1
Non-saponifiable matter.	1.36	16.14	2.67	0.93	0.56	0.93
Free fatty acids as oleic acid.....	1.6	36.8	0.8	0.25	0.25	0.37
Unsaturated fatty acids.	85.57	69.45	86.11	88.11	88.04	88.6

* The amounts of the fractions were not given in the reference. They are as follows: first fraction, 4 to 5 per cent; other fractions, 20 to 25 per cent.

of mixed triglycerides" (34), it is not to be expected that any great separation of the triglycerides will be obtained upon the distillation of an oil.

Linseed oil. The data for the molecular distillation of linseed oil by Hickman (28) are given in table 1. Since the original oil had an iodine value of 177.9 and the fractions had iodine values of 148 to 190, it is evident that some fractionation took place.

Castor oil. The data in table 2 give some of the properties of the fractions from the molecular distillation of castor oil (40). Since the fatty acids of this oil consist almost entirely of ricinoleic acid (34), it is not surprising that practically no fractionation was obtained. Disregarding the small first fraction, the iodine values varied only from 82.4 to 87.7 and the acetyl values from 122 to 133.5.

Corn oil. The distillation data in table 3 describe the first few fractions of corn oil distilled by Rawlings (39). The composition of the tri-

TABLE 2
The molecular distillation of castor oil
Rawlings (1941)

FRACTION*	IODINE VALUE	SAPONI- FICATION VALUE	PER CENT UNSAPONI- FIABLE MATTER	SAPONI- FICATION VALUE OF GLYCERIDES	IODINE VALUE OF UNSAPONI- FIABLE MATTER	IODINE VALUE OF GLYCERIDES	ACETYL VALUE
1.....	101.8	169.0	7.8	183.0	127.0	99.6	
2.....	87.7	182.0	0.25	182.5	182.5		122
3.....	85.8	182.0	Nil	182.0			124
4.....	85.6	181.5	Nil	181.5			125.5
5.....	85.2	180.5	Nil	180.5			128.5
6.....	85.0	181.0	Nil	181.0			131
7.....	82.4	180.5	Nil	180.5			133.5
Residue.....	82.8	180.5	Nil	180.5			127.5
Original.....	85.0	180.0	0.4	180.5	228.0	84.5	128.0

* The amounts of the fractions are as follows: first fraction, 4 per cent; other fractions, 12 to 15 per cent.

TABLE 3
The molecular distillation of corn oil
Rawlings (1939)

CHARACTERISTIC	ORIGINAL OIL (REFINED)	FRACTION 1	FRACTION 2	FRACTION 3	FRACTION 4	RESIDUE
Per cent distilled.....		0.01	2.58	2.73	5.05	
Distillation temperature, °C.....		180	245.	257.	280.	
Color (Evelyn L440).....	1.40	Brown solid	Brown mush	1.28	1.00	0.85
Color (Evelyn L520).....	0.23			0.242	0.085	0.201
Lovibond (yellow).....				35.	35.	50.
Lovibond (red).....				10.6	4.3	11.2
Free fatty acid (oleic), per cent..	0.033	10.6	0.95	0.20	0.10	0.02
Unsaponified matter, per cent...	1.6	64.2	15.0	5.0	2.7	0.8
Refractive index at 40°C.....	1.4678			1.4682	1.4674	1.4672
Iodine value (Wijs).....	127.0	151.4	125.7	121.3	123.6	127.6
Iodine value of fatty acids.....		115.6	121.9			
Thiocyanogen value.....	79.4			74.6	76.8	80.3
Thiocyanogen value of fatty acids.....		79.8	77.2			
Saponification value.....	190.	70.	165.	184.	188.	192.
Saponification value of glycerides.....	193.	196.	194.	194.	193.	193.
Saturated fatty acids, per cent..	7.3	(11.4)*	(14.5)			
		0.4	12.3	8.0	7.8	5.9
Oleic acid, per cent.....	35.4	(49.0)	(36.2)			
		17.5	30.7	31.1	33.4	36.8
Linoleic acid, per cent.....	54.4	(39.6)	(49.3)			
		14.2	41.8	51.6	51.8	52.2

* Values in parentheses are per cent composition of fatty acid portion.

TABLE 4
The molecular distillation of distilled soybean oil
Rawlings (1939)

CHARACTERISTIC	ORIGINAL OIL (REFINED)	FRACTION 1	FRACTION 2	FRACTION 3	FRACTION 4	FRACTION 5	FRACTION 6	FRACTION 7	RESIDUE
Per cent distilled.....		0.02	3.4	4.4	4.4	23.2	20.4	28.4	
Distillation temperature, °C.....		170-178	265-265	265-270	275	280	205	295	
Color (Ivelyn I440).....	1.35	Too dark		1.28	1.28	1.10	0.33	0.40	1.28
Color (Ivelyn L520).....	0.716	Too dark		0.328	0.172	0.059	0.019	0.023	0.810
Lovibond (yellow).....	70*			35†	35†	25†	18†	20†	95†
Lovibond (red).....	14.1*			11.2†	6.2†	3.0†	1.6†	1.7†	66†
Free fatty acid (as oleic), per cent.....	0.04	5.0	0.5	0.2	0.15	0.10	0.06	0.04	0.09
Unsaponified matter, per cent.....	0.8	74.2	10.7	1.5	0.7	0.28			
Saponification number.....	193	50	175	193	193.5	193	193	194	193
Saponification number of glycerides..	194	194	196	196	195	194	193	194	193
Refractive index at 40°C.....	1.4682			1.4673	1.4672	1.4673	1.4676	1.4674	1.4682
Iodine value (Wijs).....	134.5	150.8	131.2	127.2	127.8	129.9	131.2	132.0	137.6
Thioyanogen value.....	83.7			79.7	79.2	82.3	83.0	80.6	87.1
Iodine value of fatty acids.....	139.2		130.3	134.7	132.8	136.8	136.8	137.0	143.2
Thioyanogen value of fatty acids....	86.8		81.3	83.1	82.8	85.8	86.3	85.8	90.1
Solid fatty acids, per cent.....	12.5		16.0	14.9	15.8	12.9	12.6	12.2	11.0
Unsaturated acids (by difference), per cent.....	87.5		84.0	85.1	84.2	87.1	87.4	87.8	89.0
Oleic acid.....	29.7		23.2	26.2	29.2	30.9	31.7	31.3	30.4
Linoleic acid, per cent.....	49.4		54.8	50.1	47.7	48.4	47.7	49.4	48.0
Linolenic acid, per cent.....	8.4		6.0	6.8	7.3	7.8	8.0	7.1	10.6

* 2.5-in. column.

† 5.25-in. column.

‡ 1-in. column.

glycerides was almost exactly the same in all fractions except the first, which amounted to only 0.01 per cent in weight.

Soybean oil. The data for the almost complete distillation of soybean oil (39) are given in table 4. The lack of separation of the constituent triglycerides is shown by the fact that the iodine values for the fatty acids in the fractions range only from 132.6 to 143.2.

Cottonseed oil. Table 5 shows the data for the distillation of cottonseed oil by Riemenschneider, Swift, and Sando (41). The separation of the

TABLE 5
The molecular distillation of cottonseed oil
Riemenschneider, Swift, and Sando (1940)

FRACTION NO.	WEIGHT	SAPONIFICATION EQUIVALENT*	FATTY ACID AS OLEIC	REFRACTIVE INDEX AT 40°C.	UNSATURABLE	IODINE NUMBER	THIOCTANONUMBER	COMPOSITION		
								Linoleic acid	Oleic acid	Saturated acids
	grams		per cent	-				per cent	per cent	per cent
1.....	88.0	280.2	0.38	1.4660	5.83	100.6	57.3	50.0	16.2	33.8
2.....	93.7	284.0	0.14	1.4634	0.60	100.0	58.4	48.0	19.5	32.5
3.....	93.0	284.3	0.13	1.4635	0.34	101.2	59.3	48.3	20.1	31.6
4.....	89.0	285.2	0.11	1.4638	0.25	103.0	60.3	49.3	20.4	30.3
5.....	87.7	285.4	0.09	1.4639	0.23	103.4	60.6	49.4	20.7	29.9
6.....	93.5	284.1	0.07	1.4639	0.16	103.9	60.7	49.8	20.4	29.8
7.....	90.5	284.6	0.06	1.4641	0.16	106.1	61.3	51.7	19.1	29.2
8.....	89.5	285.0	0.05	1.4642	0.13	106.1	61.9	51.0	20.6	28.4
9.....	90.0	284.7	0.05	1.4644	0.14	107.9	62.2	52.7	19.2	28.1
10.....	93.0	284.0	0.06	1.4645	0.17	109.3	63.5	52.8	20.6	26.6
11.....	91.0	286.3	0.06	1.4646	0.13	110.3	63.9	53.5	20.4	26.1
12.....	92.0	286.6	0.05	1.4648	0.09	111.9	65.2	53.9	21.5	24.6
13.....	89.0	286.6	0.05	1.4650	0.07	114.4	66.3	55.5	21.2	23.3
14.....	87.5	287.3	0.06	1.4654	0.11	116.6	67.9	56.2	22.3	21.5
15.....	85.5	290.1	0.06	1.4661	0.13	120.9	70.2	58.5	22.7	18.8
Residue.....	43.4	290.0	0.09	1.4706	0.68	118.9	67.2	59.6	18.0	22.4
Original oil..	1417.0	285.8	0.10	1.4645	0.49	108.3	62.3	53.1	18.9	28.0

* Corrected for unsaponifiable material.

triglycerides is somewhat better than that in the case of soybean oil, but even so the iodine values of the fractions range only from 100 to 120.9.

Menhaden oil. The distillation data for menhaden oil shown in table 6 were obtained by Cawley, Barnitz, and Jackson (8). Since there are present in fish oils considerable amounts of each of the fatty acids from C₁₄ to C₂₂, it would be expected that some degree of fractionation of the fish-oil triglycerides could be obtained. The fact that the iodine values of the distillates ranged from 142.5 to 244 shows that this is indeed the case. The molecular distillation of fish-liver oils may prove to be of

considerable value for the determination of the triglyceride structure of these extremely complex materials.

TABLE 6
Molecular distillation of menhaden oil
Cawley, Barnitz, and Jackson (1941)

	FRACTION*	FRACTION	FRACTION	FRACTION	FRACTION	FRACTION
Acid value.....	1.45	7.14	0.09	0.05	0.08	0.20
Iodine value.....	183.	142.5	157.5	173.5	202.5	244.
Saponification value.....	180.3	181.9	184.6	181.6	176.2	171.3
Refractive index, n_D^{25}	1.4794	1.4744	1.4758	1.4779	1.4818	1.4876
Mean unsaturation (double bonds per triglyceride molecule).....	6.73	5.19	5.66	6.34	7.63	9.45

* Each fraction amounted to 20 per cent of the original oil in weight.

TABLE 7
Vacuum distillation of babassu nut oil
Bömer and Hüttig (1938)

	ORIGINAL OIL	FRACTION 1	FRACTION 2	RESIDUE
Per cent cut.....		5.2	77.2	17.0
Temperature, °C.....		To 150	150-295	
Melting point, °C.....	25	25.5	26.8	17.0
Saponification value.....	251.1	(Mainly free	256.0*	222.8*
Iodine value.....	15.6	fatty acids)	8.8*	44.7*

* Of triglycerides.

TABLE 8
Vacuum distillation of coconut oil
Bömer and Baumann (1920)

	ORIGINAL OIL	DISTILLATE	
Per cent cut.....		87.7	12.3
Temperature, °C.....		250-285	
Melting point, °C.....	26.8	25.0	32.5
Saponification value.....	259.7	263.7	228.5
Iodine value.....	4.6	1.85	23.5

Coconut and babassu oil. The fats from the nuts of the *Palmae* have considerable amounts of each of the fatty acids containing from eight to eighteen carbon atoms. Bömer and Hüttig (4) were able partially to

distill babassu nut oil, and Bömer and Baumann (3) were able partially to distill coconut oil in a high-vacuum (not a molecular) still. The distillation data are given in tables 7 and 8. Waterman and Rijks (48) were able to distill coconut oil completely in an improved high-vacuum still to obtain the fractions described in table 9. These distillations show that the glycerides of the saturated fatty acids of low molecular weight distill first, and that the glycerides containing oleic acid distill last.

TABLE 9
Vacuum distillation of coconut oil
Waterman and Rijks (1926)

	ORIGINAL OIL	FRACTION 1	FRACTION 2	FRACTION 3	FRACTION 4
Per cent cut.....		30.0	17.5	27.5	21.5
Temperature of distillation, °C....		206-223	223-229	229-231	231-254
Acid value.....	0.945	1.4	0.9	0.9	0.27
Saponification value.....	256.	280.	274.	262.	237.
Iodine number.....	8.2	2.4	1.5	4.3	23.3

E. Conclusions

The distillation data given for the vegetable oils show that molecular distillation will remove the free fatty acids and the unsaponifiable constituents of the oil with considerable efficiency. This method of concentration is of especial value when the substance studied may be altered by saponification. Such substances are vitamin K, sterol esters, and possibly others. The present types of molecular stills will be of little value for the separation of the triglycerides of vegetable oils.

The molecular distillation of fish-liver oils for the concentration of the unsaponifiable constituents has proved to be of great value, because vitamins A and D are recovered in their natural esterified state. Furthermore, the separation of the triglycerides of fish oils by molecular distillation takes place to an extent great enough to be of scientific interest.

Since so few oils have been carefully examined by molecular distillation, the above statements may have to be modified. Many laboratories interested in the study of oils have only recently acquired molecular stills, and it is expected that much new information will soon be available on this subject. Furthermore, the scientific and commercial rewards for devising equipment which will better separate the triglycerides may lead to the development of molecular stills or combinations of stills which will have considerably enhanced powers.

V. THE MOLECULAR DISTILLATION OF BODIED OILS

Although the constituents of heat-bodied oils can scarcely be considered, to be "natural components" of oils, the work on the molecular distillation of such heat-bodied oils should be mentioned in this article. Waterman and Oosterhof (46) found that stand oil with a viscosity of 55 poises gave 32 per cent of a distillate which had poor drying qualities and deposited stearin on standing. Its molecular weight was 757, showing that it consisted of unpolymerized glycerides. The undistillable residue (68 per cent) had a viscosity of 379 poises and a molecular weight of 3463. It remained clear on standing and had *better* drying qualities than the original stand oil.

Apparently, when an oil is heat-bodied, the triglycerides containing the reactive unsaturated fatty acids are linked together through condensation of these acids. The triglycerides containing saturated fatty acids and unreactive unsaturated fatty acids do not polymerize and may therefore be distilled from the mixture. Processes for the removal of these unreactive (and hence undesirable in a drying oil) triglycerides from bodied linseed and Chinawood oils have been described by Oosterhof, van Vlodrop, and Waterman (38).

Fawcett and Walker (18, 19) have found that good drying oils may be made in a similar manner by distilling the unpolymerized glycerides from bodied fish oils.

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LOW-TEMPERATURE CRYSTALLIZATION OF THE FATTY ACIDS AND GLYCERIDES¹

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I. INTRODUCTION

Crystallization has been used in the study of the composition of the fats and oils since early in the nineteenth century. The objective of this method of investigation was the isolation of pure naturally occurring glycerides, thus affording definite information on glyceride structure, but the isolation of pure compounds was found to be extremely difficult because of the complexity of composition of the component glycerides of the fats. Only three reports have described the application of very low temperatures to the partial separation of the liquid glycerides of the fatty oils; of these, two have appeared within the past year. As applied to the fatty acids,

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crystallization has been used mainly as a method of final purification of the higher saturated acids and certain solid unsaturated acids. Owing to mixed-crystal formation, it was often necessary to carry out such crystallizations almost a prohibitive number of times. Only within the past five years have serious attempts been made to employ the method in the isolation of the liquid unsaturated acids. In this work temperatures as low as $-75^{\circ}\text{C}.$ were used in order to reduce the solubilities to values which are practical for crystallization methods and to work at temperatures at which the unsaturated acids are crystalline solids.

In the present discussion previous work on the crystallization of both fatty acids and the fats has been reviewed. More emphasis has been placed, however, on recent applications to fatty acid chemistry, because the low temperatures employed are somewhat unusual in this field of investigation and the possibilities of the method seem to be almost without limit in the study and separation of these compounds. Less attention has been devoted to the application of the method to the fats and oils, because most of the work reported in the literature has employed temperatures above $0^{\circ}\text{C}.$ and because the separations which were achievable by this technic have been disappointing.

II. THE APPLICATION OF LOW-TEMPERATURE CRYSTALLIZATION TO THE SEPARATION OF THE FATTY ACIDS

Discussion of this subject will be confined primarily to work reported during the past five years on certain of the unsaturated acids which occur as major component acids in fats and oils. This work has included the separation and isolation of such acids as oleic and linoleic and the application of crystallization to the partial purification of certain other unsaturated acids. Further, it has applied the technic to the separation of the saturated and unsaturated acids and to the separation of isomeric linoleic and linolenic acids in mixtures resulting from the debromination reaction.

Generally, the naturally occurring unsaturated acids are liquid at room temperature; as a rule, they have much lower melting points than the saturated acid of the same series. Most of the unsaturated acids are infinitely soluble in organic solvents at temperatures above $0^{\circ}\text{C}.$, while the saturated acids are sparingly soluble at this temperature in such solvents as acetone, petroleum ether, and alcohol. In other solvents, such as ether and chloroform, the solubility differences are not so marked.

Several factors have contributed to the slow progress in the application of crystallization methods to the separation of the fatty acids, and more particularly the unsaturated acids. In the first place, the impression has been general that fatty acids tend to exist in solution in an associated, dimeric state and that they crystallize as such from solutions. Thus with

mixtures, mixed crystals are formed. In the second place, the unsaturated acids have been regarded as oily liquids which are not adapted to this type of procedure. In the third place, the general lack of low-temperature apparatus in most laboratories has not encouraged research in this field.

The low melting points and almost infinite solubilities of the common unsaturated acids at temperatures above 0°C. led to the development of both physical and chemical methods of separation in which higher melting, less soluble derivatives were used. Among the physical methods, fractionation of the methyl esters affected the separation of acids of different carbon series. The use of soaps instead of the free acids served to raise the melting points and lower the solubilities, so that certain separations could be made at temperatures of 0°C. and higher. The most widely used soap method is the official lead soap-ether method (7) or the Twitchell lead soap-alcohol modification (9, 116). The chemical methods have employed high-melting derivatives of the fatty acids, such as the bromine addition compounds and the hydroxy acids. The disadvantages of these chemical methods are familiar to those who have used them. From the work which is reviewed below, it is evident that crystallization procedures for separating the fatty acids from one another and for actually preparing them in the pure state constitute the simplest and most direct methods so far available.

A. The isolation of oleic acid

At room temperature oleic acid is an oily liquid and is infinitely soluble in most organic solvents. Bertram (10) seems to have been the first investigator to crystallize oleic acid from an organic solvent. After the application of certain soap procedures designed to remove saturated acids, he cooled a mixture of equal volumes of oleic acid and acetone to -10° to -15°C. The crystals were further crystallized twice from the same solvent, thereby removing, as he claimed, traces of remaining saturated acids and linoleic acid. Raymond (103) used alcohol at -15° to -25°C. as a crystallizing medium. Both investigators employed concentrated solutions instead of much lower temperatures to reduce solubilities to practical levels.

In 1937 Brown and Shinowara (33) reported the preparation of oleic acid by direct crystallization of the fatty acids of olive oil and without the use of any soap separations. In this work the assumption was made that the fatty acids of olive oil belong to three solubility groups: the higher saturated acids, oleic acid, and linoleic acid. Such an assumption was necessary because of the complete lack of solubility data on oleic and linoleic acids. A solution of 225 g. of olive oil fatty acids in 3450 cc. of acetone was cooled to -20°C. overnight and filtered. The filtrate was cooled to

-60°C . in a dry ice-alcohol bath and again filtered. The resulting crystal fraction was subjected to three further crystallizations at -60°C ., each time from 2000 cc. of acetone. By this procedure the more soluble linoleic acid was removed in the several filtrates. After these four crystallizations at -60°C ., the product was made up to 1250 cc. with acetone and cooled slowly to the first appearance of crystals, about -35°C . Although this crystal crop was largely oleic acid, it contained some additional saturated acids which had not been removed by the first separation at -20°C . The product remaining in the filtrate was oleic acid, the analytical constants of which were identical for practical purposes with those of another specimen obtained by a similar procedure which included, however, six crystallizations at -60°C . At the time this work appeared, these specimens of oleic acid compared favorably with several of the best preparations previously reported, with the possible exception of the one reported by Bertram, who claimed a purity of 99.5 per cent. The actual purity of these crystallization products, in view of later work, was probably of the order of 96 per cent. Nevertheless the simplicity of this method in comparison with the combination of repeated soap separations, distillation, and crystallization previously used has recommended it to other investigators.

Hartsuch (54) in 1939 made a detailed study of the fractions which appeared during a similar fractionation and verified the fact that linoleic acid is easily removed by crystallization at -60°C . In a series of four crystallizations at this temperature from a 10 per cent solution in acetone, the iodine numbers of the consecutive filtrates were 144.5, 119.8, 92.5, and 89.0, respectively. That of the final crystal fraction was 87.6. Hartsuch removed the remaining saturated acids in part by the use of a modified Twitchell separation but mainly by a careful fractional distillation of the acid. His yield of oleic acid was relatively small; the claimed purity was 97.8 per cent.

J. C. Smith (113) prepared oleic acid by the following procedure: The methyl esters of a "high grade commercial" oleic acid were fractionally distilled to remove palmitic and some "stearic acid". The acids prepared from the resulting mixture of methyl oleate and linoleate were then crystallized from acetone solution to a constant melting point, 13.36°C . Stearic acid prepared from this product by catalytic reduction showed a depression of the melting point indicating the presence of less than 0.2 per cent of palmitic acid. Smith described several types of low-temperature filtration apparatus.

Five months later Wheeler and Riemenschneider (118) reported a detailed procedure for preparing very pure methyl oleate. Since the crystallization procedure failed to remove the palmitic acid entirely, they first made the pure C_{18} methyl esters from olive oil acids by a very careful

fractional distillation, and applied crystallization procedures afterwards. The following steps were described: (1) Fractional distillation of the methyl esters of the mixed acids of olive oil to remove palmitic and lower esters. (2) Removal of most of the linoleic ester (in the filtrate) by crystallization from acetone (15 cc. per gram) at -60°C . (3) Precipitation of the bulk of the saturated esters from acetone (10 cc. per gram) at -37°C . and removal by filtration. (4) Further elimination of methyl linoleate by two crystallizations from acetone (15 cc. per gram) at -60°C . (5) Fractional distillation *in vacuo* to remove small amounts of remaining methyl palmitate. (6) Two crystallizations (at -65°C .) from redistilled petroleum ether (6 cc. per gram; b.p. $30-45^{\circ}\text{C}$.) to remove the last traces of linoleate. The iodine number was not changed by the last two crystallizations. The purity was more than 99.6 per cent.

We have recently prepared very pure specimens of methyl oleate and oleic acid by a similar procedure (47), except that the C_{18} methyl esters of olive oil were repeatedly crystallized from methyl alcohol. These esters were composed of approximately 14 per cent linoleate, 83.5 per cent oleate, and 2.5 per cent stearate. A solution of 200 g. of these esters in 4 liters of methyl alcohol was cooled to -60°C . and filtered. The crystal fraction was dissolved in enough methyl alcohol to bring it up to the volume of solution employed in the first crystallization. Five successive crystallizations, each time from the same concentration of solution, were thus carried out. The filtrate fractions decreased from 15.0 g. in the first filtrate to 0.6 g. in the fifth filtrate. The first filtrate was made up of a mixture of methyl oleate and methyl linoleate containing about 88.7 per cent of methyl linoleate, calculated from the iodine number as a binary mixture of oleate and linoleate. The final crystal fraction contained 3.3 per cent of methyl stearate, which was almost completely removed in one step as follows: 100 g. of the oleate-stearate mixture in 4 liters of methyl alcohol was placed in the cold room at -22.5°C . for 2 days. A crop of large flat crystals came down and was removed. The yield was 3.2 g.; the iodine number of the product was 1.5. This was nearly pure methyl stearate. This experiment is reported here because it demonstrates an almost unbelievably sharp separation between two esters of the same carbon series. It is apparent that mixed-crystal formation under the conditions described is negligible.

B. The isolation of linoleic acid

The classic and only method available previously for the preparation of pure linoleic acid has been by the debromination of pure tetrabromostearic acid, obtained by bromination of the fatty acids of those semi-drying oils which do not contain linolenic acid (105). The debromination method

yields a product which is pure as evaluated by iodine number, but it is a wasteful method because more than half of the original acid is lost in the form of soluble, isomeric bromides. Furthermore, the method introduces the question of isomerism, both of the bromides and of the product (30, 96).

In 1935, in connection with some work on linoleic acid, it occurred to the writer that it should be possible to separate this acid from the oleic and saturated acids of cottonseed oil by precipitating the latter acids from solutions in organic solvents at very low temperatures. In our original report (34) of this work we described the separation of the saturated and unsaturated acids of this oil by cooling solutions in acetone and certain other solvents to -20°C . The unsaturated acids thus obtained were fractionally crystallized from acetone and methyl and ethyl alcohols. Typical results are shown in table 1. In this experiment 1 liter of methyl alcohol solution containing 100 g. of unsaturated acids was cooled, and

TABLE 1

Fractional crystallization of the unsaturated fatty acids of cottonseed oil from methyl alcohol

FRACTION	TEMPERATURE	WEIGHT	IODINE NO.	LINOLEIC ACID
	$^{\circ}\text{C}$.	grams		per cent
Crystals.....	-50	25	134.8	50
Crystals.....	-55	16	159.2	76
Crystals.....	-60	28	157.7	75
Crystals.....	-65	15	168.2	86
Filtrate.....	-65	16	156.5	73

successive crystal fractions were taken off at progressively lower temperatures.

By various modifications of this procedure, crystallization fractions containing from 85 to 93 per cent linoleic acid were isolated from corn oil. Attempts to crystallize methyl esters gave less encouraging results. In a later study Brown and Frankel (30) crystallized the fatty acids of corn oil and obtained a good yield of 93.5 per cent linoleic acid in the fraction crystallizing between -50° and -70°C . Preparation of acid of this purity has made possible a comparison of the properties of linoleic acid, prepared by direct crystallization, with those of the purer acid obtained by debromination. The probable identity of the two products was thus established.² In a very recent report Brown and Frankel (31) have described the preparation of pure linoleic acid by a crystallization procedure.

² In a recent report from this laboratory (96), described later, debromination linoleic acid has been shown to contain about 12 per cent of an isomer, probably of the *cis-trans* type. The crystallization acid appears to be identical with the major

Platz and Steenbock (101) crystallized the acids of wheat germ oil from acetone. Fractions appearing at -45° , -50° , -55° , and -75°C. were converted into ethyl esters for feeding purposes in a study of rat acrodynia. The iodine numbers of the respective fractions were 117, 141, 149, and 153. The -75°C. fraction, containing about 86 per cent of ethyl linoleate, was found to be of about equal biological activity to ethyl linoleate obtained by debromination. G. O. Burr and coworkers (37) at Minnesota have found our linoleic acids obtained by crystallization to be of the same activity in curing the fat deficiency syndrome as debromination linoleic acid.

C. The use of low-temperature crystallization in the isolation of acids containing three and four double bonds

As in the case of linoleic acid, the usual method for the preparation of pure linolenic and arachidonic acids ($\text{C}_{18}\text{H}_{30}\text{O}_2$ and $\text{C}_{20}\text{H}_{32}\text{O}_2$) has been by the debromination of hexabromostearic and octabromoarachidic acids, respectively. In both cases losses through the formation of soluble, isomeric bromides are considerably greater than with linoleic acid.

Shinowara and Brown (111) attempted the isolation of linolenic acid from linseed and perilla oils. The problem with both oils is more complicated than the isolation of linoleic acid, because, in addition to the presence of the desired linolenic acid (40 to 50 per cent), three additional solubility classes of acids are present: the saturated acids, oleic acid, and linoleic acid. Thus, while it was comparatively easy to remove most of the saturated acids by crystallization from acetone at -20°C. , it was more difficult to remove all of the oleic acid, and still more difficult to separate the linoleic and linolenic acids. Solubility differences between the latter two are relatively small. The fractionation was carried out, starting with 500-g. portions of the fatty acids made up to 6 liters with acetone. Data are given in table 2.

Approximate calculations of the solubilities of linoleic and linolenic acids may be made from the data in table 2. At -75°C. the filtrates were mixtures of about one part linoleic acid with three parts of linolenic acid. Assuming the volume of the filtrate at -75°C. to be about 4500 cc. (about one-quarter of the original 6 liters of solution has been removed with the four crystal fractions), the solubility of linoleic acid is 3.3 g. per liter of solution and of linolenic acid 10 g. per liter. At -60°C. the filtrate amounted to about 5000 cc., and there remained in this solution about 170 g. of acids containing about one-third linoleic acid and two-thirds linolenic

constituent of the debromination acid. In the present discussion the expression "crystallization acid" refers to a product prepared exclusively by crystallization methods; "debromination acid" refers to an acid prepared by reduction of the addition compounds of bromine and the unsaturated acids.

acid. The solubilities of the two acids are 10 and 20 g. per liter of solution respectively. It should be emphasized again that these are rough estimates only, since in this early work accurate measurement of filtrate volumes was not made, and no account has been taken of the presence of oleic acid in these filtrates.

Several other attempts were made without success to increase the purity by varying the conditions of crystallization, the purity of the products ranging from 70 to 77 per cent. Later it was found that repeated crystallization of these products from petroleum ether removed additional amounts of linoleic acid. Thus, six successive crystallizations of 69 per cent linolenic acid from this solvent at $-60^{\circ}\text{C}.$ gave our best product,—namely, an acid of 88 per cent purity. From the preceding data (table 2) it was noted that the solubility ratio of linolenic acid to linoleic acid in acetone was 3 to 1 at $-75^{\circ}\text{C}.$ and 2 to 1 at $-60^{\circ}\text{C}.$ The improvement in purity as a result

TABLE 2
Fractional crystallization of the fatty acids of linseed and perilla oils

FRACTION	TEMPERATURE	LINSEED OIL			PERILLA OIL		
		Weight	Iodine No.	Linolenic acid*	Weight	Iodine No.	Linolenic acid*
	$^{\circ}\text{C}.$	grams		per cent	grams		per cent
I.....	-23	65	7.8		40	63.9	
II.....	-45	124	165.2		20	142.3	
III.....	-60	137	217.6	38	235	220.3	41
IV.....	-75	118	242.1	65	130	242.8	66
Filtrate.....	-75	56	252.9	77	65	249.1	74

* Calculated from the iodine number as a binary mixture of linoleic and linolenic acids.

of recrystallization from petroleum ether at $-60^{\circ}\text{C}.$ may have been due to a more favorable solubility ratio, whereby crystal fractions at $-60^{\circ}\text{C}.$ were richer in linolenic acid.

Apparently crystallization procedures have not been applied to other trienoic acids, except in the case of eleostearic acid. Schumann (108) has reported a crystallization method for the estimation of this acid. A detailed procedure has been described by Ku (90), who employed the crystallization of tung oil acids from 76 per cent alcohol at $0^{\circ}\text{C}.$ It is to be noted that both α - and β -eleostearic acids have relatively high melting points; hence they tend to behave in solubility like the higher saturated acids. Ku reported the solubility of several fatty acids. Some of his data are included in table 4. Ku (90) and Thomas and Thomson (114) prepared their eleostearic acid by recrystallization from alcohol.

The only attempt to isolate a tetraunsaturated acid by low-temperature

crystallization was reported by Shinowara and Brown (112) in 1940. The problem in this study was to separate arachidonic acid from a mixture of the acids of beef adrenal phosphatides. In addition to 20–30 per cent of arachidonic acid, these phosphatides contain oleic and saturated acids. The principal difficulty is due to the fact that both arachidonic acid and its methyl ester remain in the filtrate. Methyl arachidonate is a liquid at the temperature of dry ice. The acid melts at -49.5°C . Both are very soluble in organic solvents, even at -75°C . By the use of either the free acids or the methyl esters it was not possible to prepare a product containing above 75 per cent of arachidonic acid. In one experiment, for example, 175 g. of 56 per cent methyl arachidonate in 800 cc. of acetone, cooled to -75°C ., gave 100 g. of filtrate esters, containing only 70 per cent of methyl arachidonate. The principal contaminant here is methyl oleate, which should have been rather completely removed by this treatment, but which was apparently much more soluble in the presence of large amounts of methyl arachidonate. Advantage was finally taken of the difference in boiling points to prepare methyl arachidonate of 95 per cent purity by careful distillation.

Any application of low-temperature crystallization to the preparation of the highly unsaturated acids of fish oils will apparently be limited by the considerations described above. It is likely that the solubilities of these acids with four, five, and six double bonds and of carbon series from C_{18} to C_{22} will be too great even at temperatures below their melting points to make it possible to isolate them in the form of crystal fractions. Actual crystallization is necessary to attain any high degree of purification.

D. The preparation of pure methyl ricinoleate

The isolation of several specimens of methyl ricinoleate of very high purity by low-temperature crystallization of the methyl esters of castor oil was reported in 1940 by Brown and Green (32). Both acetone and methyl alcohol were used as solvents, the latter being somewhat the better. The crystal fraction coming down between -40° and -65°C . from a 5 per cent solution in methyl alcohol was 98 per cent pure, as evaluated by the acetyl number. This degree of purity, as a result of a single crystallization, is believed to be due to the chemical dissimilarity of methyl ricinoleate from the other esters present,—oleate, linoleate, and saturated esters,—as a result of which the formation of mixed crystals is very slight.

E. Preliminary observations on chaulmoogric and erucic acids

Our work on these acids is still not complete. These brief descriptions are included to illustrate the application of the method in the partial purification of these solid unsaturated acids. ♣

The high melting points of chaulmoogric and hydnocarpic acids, 71° and 59°C. , respectively, and the low (not reported) melting point of gorlic acid, the three principal acids of chaulmoogra oil, suggest that crystallization from organic solvents might afford a convenient fractionation procedure. Preliminary work (11) shows that, starting with the 5 per cent solution of the fatty acids of chaulmoogra oil in acetone, chaulmoogric acid, along with the saturated acids, comes down principally in the crystal fraction at -20°C. , hydnocarpic acid in the crystals at -50°C. , and that gorlic acid remains in the filtrates at -50°C. Gorlic acid is still quite soluble in acetone at -60°C.

Erucic acid, which makes up 40 to 50 per cent of the acids of rape seed oil, has been prepared by Holde and Wilke (76) from the acids of the ether-insoluble lead soaps by recrystallization from alcohol in order to remove saturated acids. In preliminary experiments in our laboratory (6) we have found that erucic acid comes down from an acetone solution in the -20°C. crystal fraction along with the saturated acids. When this product in 5 per cent solution in acetone is cooled to about -8°C. , a small crop of crystals, mainly saturated acids, forms and is removed. On cooling further to -20°C. , the crystal fraction is crude erucic acid (iodine number 72 and molecular weight 336; theoretical values, 74.5 and 338, respectively).

It is likely that other solid unsaturated acids, such as isoöleic acid, may be conveniently separated from the saturated and liquid unsaturated acids by proper choice of solvent and temperature. Elaidic acid, the *trans*-isomer of oleic acid, may be easily separated from oleic acid by crystallization.

F. The determination of saturated acids

Lewkowitsch quotes Fachini and Dorta (45) as having first tried separating saturated and unsaturated acids by cooling petroleum ether solutions to -40 to -45°C. In our first paper (34) on low-temperature methods we described the separation of the saturated and unsaturated acids of cottonseed oil by cooling solutions of the acids in methyl or ethyl alcohol, acetone, and petroleum ether to -20°C. ³ Five out of seven trials with these solvents gave saturated acids of iodine number from 3.4 to 7.2. Ten to 12 per cent solutions were employed and the crystals were washed with cold solvent. We suggested then that the method might be used as a substitute for the official lead soap-ether or -alcohol procedures. Such a method could not be successful, however, either if association in solution between the two classes of acids were appreciable, or if they tended to form

³ This method has been used by Dermer and Crews (41) in their study of the oil of *Sapindus Drummondii*.

mixed crystals. To minimize these effects, it seemed likely that solutions of lower concentration than 10 per cent would have to be used. On the other hand, with very dilute concentrations the solubility of the saturated acids would be an important error.

An excellent study of a crystallization method for the determination of the saturated acids in soybean oil has been described by Earle and Milner (43). In their method a 5-g. sample of the mixed fatty acids is dissolved in 50 cc. of acetone and cooled to -40°C . The crystals are filtered off and twice crystallized from the same volume of solvent at the same temperature. Results by their method agreed well with those they obtained by an oxidation procedure (Bertram's) as described by Pelikan and Von Mikusch (99) and were about 2 per cent higher than results by the Twitchell method. The liquid acids obtained by the Twitchell procedure were shown to contain long-chain saturated acids when examined by both of the other procedures. Earle and Milner stated that their method had been used with success on one specimen of cottonseed oil, but that it would probably have to be modified for use with highly unsaturated oils, such as perilla oil or for oils such as olive oil, which is high in oleic acid. Incidentally, they found the solubilities of stearic, palmitic, and myristic acids in acetone at -40°C . to be 1, 8, and 75 mg. per 100 cc., respectively.

We have analyzed eight specimens of fats and oils (102), including human fat, lard, beef tallow, cottonseed oil, and olive oil by a single crystallization procedure. A 20-g. sample of the acids is dissolved in 400 cc. of acetone and allowed to stand at -20° to -25°C . overnight. The crystals are filtered on a small Büchner funnel and washed several times with cold solvent. The crystal and filtrate fractions are quantitatively recovered and weighed. The results by this method were compared with those obtained by the lead soap-ether procedure. The iodine numbers of the saturated acids by the latter method ranged from 3.26 to 31.40, while those by the former gave values of 0.28 to 6.40. However, the contents of saturated acids by the crystallization method were generally 10 to 30 per cent lower than those by the soap method. The low iodine numbers by the former method indicate that mixed-crystal formation is relatively slight under these conditions, but, on the other hand, it seems likely that for quantitative results somewhat lower temperatures will have to be employed in order to reduce the solubilities of the saturated acids, especially myristic and palmitic. A comprehensive study of the application of this method to the analysis of a wide variety of fats and oils is desirable. It should be possible to work out a "single crystallization" method for most classes of fats and oils, thus avoiding the several crystallizations of the Earle and Milner method.

A quick low-temperature method for separating high- and low-titer fatty

acids has been reported recently by DeGray and DeMoise (40). The method involves cooling 1 to 2 per cent solutions of acids in petroleum ether to -45°C . or below. With one crystallization they claimed an efficiency of separation of 95 per cent. They described a jacketed funnel for low-temperature filtration.

G. The application of low-temperature crystallization to the separation of isomeric unsaturated acids

For many years it has been known that the *cis-trans* isomers, oleic and elaidic acids, may be separated by crystallization. The ease with which elaidic acid may be purified has been the basis of its use by Kass and co-workers as a standard for the determination of the thiocyanogen number

TABLE 3
Separation of debromination linoleic acid by crystallization at -60°C .

	WEIGHT	TETRA-BROMIDE NO.	n_{20}°	ISOMERIC ACID	IODINE NO.
	grams			grams	
Original acid*.....	170	90.6	1.4699	20.6	181.2*
Filtrate I.....	8.8	44.8	1.4700	5.0	179.0
II.....	7.4	45.8	1.4700	4.1	177.5
VIII.....	3.5	66.7	1.4700	1.2	179.5
X.....	5.2	93.9	1.4699	0.5	181.1
XII.....	5.1	97.9	1.4700	0.25	180.7
Final crystals†.....	92	102.9	1.4699	0.0	181.0

* Melting point -8.8 to -7.1°C .

† Melting point -5.2 to -5.0°C .

of the 9,10-octadecenoic acids (78) and, by hydrogenation, as a method for the preparation of pure stearic acid (77). From structural considerations, mixed-crystal formation between oleic and stearic acids and oleic and elaidic acids,—in fact, between *cis-trans* isomers generally,—should be slight. Crystallization, therefore, should constitute an excellent method for separating isomers of this type.

Very recently we have succeeded in resolving debromination linoleic acid into two acids by repeated crystallization at -60°C . (96). The original debromination acid, containing about 12 per cent of an isomeric linoleic acid, in a 2 per cent solution in petroleum ether was crystallized twelve times from constant volumes of solution, the filtrates and final crystal fractions being recovered and analyzed. The results in part are shown in table 3.

In table 3 the amounts of isomeric acid were calculated on the assumption that its tetrabromide number is zero and that of true linoleic acid is 102.9. Filtrate I above is a 60-40 mixture of the isomeric acid with linoleic acid. Assuming the filtrate to be saturated with both acids, their relative solubilities are 3:2, provided also that at this high dilution mutual solubility effects are not important. Each of the twelve filtrates was saturated with linoleic acid, but each contained progressively less of the contaminating impurity. Thus, at the expense of about half of the wanted material, the impurity was practically quantitatively removed. Equally satisfactory results were obtained in the separation of debromination linolenic acid.

H. Discussion

From the preceding review of work already accomplished, it has been shown that low-temperature crystallization is a useful procedure for the separation and purification of fatty acids, especially the unsaturated acids. In the temperature range from 0° to -75°C., the solubilities of the common unsaturated acids vary from infinite solubility to one of the order of less than 0.1 per cent, a value comparable with those of the higher saturated acids at 20° to 0°C. Further, oleic, linoleic and linolenic acids, usually regarded as oils, are crystalline solids at low temperatures. Usually, but not always, crystal crops of these acids filter with great ease, and hence are well adapted to laboratory manipulation.

The possibilities of the crystallization procedure as a technic of research on the fatty acids are almost without limit. So far only a beginning has been made in this field. The greatest need at the present time is for accurate data on the solubilities of the acids in a large series of organic solvents and over a range of temperatures down to the lowest limit of dry ice. Such a study has been under way in our laboratory for the past year, but the difficulties are very great, more particularly the preparation of fatty acids of undoubted purity, and the development of low-temperature apparatus which can be held constant at the very low temperatures long enough to prevent supersaturation. We hope to have a preliminary report on this work soon.

In the meantime, it has been possible from the data above to make a number of *approximate* calculations of solubilities at the very low temperatures. These calculated values, along with the more important previously reported solubilities of myristic, palmitic, and stearic acids, are summarized in table 4.

Two important limiting factors in crystallization procedures are molecular association (compound formation in solution) and mixed-crystal formation. Waentig and Pescheck (117) in 1919, in a study of the mutual

solubility of the fatty acids, observed that palmitic acid is two and one-half times as soluble in carbon tetrachloride containing lauric acid as in

TABLE 4
Solubilities of the fatty acids

ACID	SOLVENT	TEMPERATURE, °C.	SOLUBILITY	REF- ER- ENCE
Myristic acid. . .	Petroleum ether	0	More than 1 per cent	(45)
	Petroleum ether	-18 to -20	0.092 g. per 100 cc. solvent	(45)
	Acetone	-40	0.075 g. per 100 cc. solvent	(43)
Palmitic acid. . .	94.4% alcohol	0	1.298 g. per 100 cc. solution	(55)
	100% alcohol	0	1.45 g. per 100 g. solvent	(106)
	95.0% alcohol	0	0.56 g. per 100 cc. solvent	(88)
	100% alcohol	10	2.8 g. per 100 cc. solution	(46)
	Petroleum ether	0	0.2578 g. per 100 g. solvent	(45)
	Petroleum ether	-18 to -20	0.0291 g. per 100 g. solvent	(45)
	90.0% alcohol	0	0.45 g. per 100 g. solution	(90)
	Acetone	-40	0.008 g. per 100 cc. solvent	(43)
Stearic acid.	94.4% alcohol	0	0.155 g. per 100 cc. solution	(55)
	100% alcohol	0	0.37 g. per 100 g. solvent	(106)
	100% alcohol	5	0.51 g. per 100 g. solvent	(106)
	100% alcohol	10	1.10 g. per 100 g. solvent	(106)
	95.1% alcohol	0	0.1139 g. per 100 cc. solvent	(44)
	100% alcohol	10	0.90 g. per 100 g. solvent	(46)
	90.0% alcohol	0	0.88 g. per 100 g. solution	(90)
	Petroleum ether	0	0.047 g. per 100 g. solvent	(45)
	Petroleum ether	-18 to -20	0.010 g. per 100 g. solvent	(45)
	Acetone	-40	0.001 g. per 100 cc. solvent	(43)
Oleic acid.	76.0% alcohol	0	28 g. per 100 g. solution	(90)
Methyl oleate. . .	Methyl alcohol	-22.5	Over 25 g. per liter	(47)
	Methyl alcohol	-60	0.15 g. per liter	(47)
Linoleic acid. . . .	Acetone	-60	About 10 g. per liter	(96)
	Acetone	-75	About 3.3 g. per liter	(96)
	Petroleum ether	-60	About 1.6 g. per liter	(96)
Linolenic acid. . .	Acetone	-60	About 20 g. per liter	(96)
	Acetone	-75	About 10 g. per liter	(96)
	Petroleum ether	-70	About 1.0 g. per liter	(96)

the pure solvent. The formation of easily soluble compounds containing both acids was assumed to take place. Similar compound formation was observed in chloroform, benzene, toluene, and nitrobenzene, but not in

alcohol, ether, or ethyl acetate. Association tends to increase the solubility of the more insoluble constituent, and would be likely to increase the tendency to mixed-crystal formation. Other studies in this field have been made by Francois (50), Boutaric and Roy (26), and Broughton (29). Very recently, Brocklesby (28) in a study of stearic, oleic, and linoleic acids has shown that association decreases with increase in unsaturation.

The modern view of the crystal structure of the saturated acids is that the crystal layers are made up of units of two molecules of acids, bound together by residual valences at the carboxyl groups (48, 49, 58, 97, 98, 100, 115). The tendency to form mixed crystals is the greater, the more nearly alike the fatty acids, and is marked with the higher saturated acids. Bruni and Gorni (35, 36) and Mascarelli and coworkers (92-95) have shown, on the other hand, that such naturally occurring *cis*-forms as oleic and erucic acids do not form solid solutions with their respective saturated acids, stearic and behenic, while the *trans*-forms do form such solutions. Smith (113) found no evidence of compound formation between oleic and stearic acids. Many of the separations described above were so clear-cut that it seems likely that mixed-crystal formation under the conditions studied is not an important item in the separation of the saturated from the unsaturated acids and in the separation of the several classes of the unsaturated acids from each other.

Another important difficulty in crystallization procedures is contamination of the crystal fractions with mother liquor and, as a consequence, with filtrate acids. With the cottonseed oil acids the unwashed crystals, even with efficient suction filtration, contained four to five times their weight of solvent. This represents a tremendous contamination of the true crystal fraction with actual filtrate acids. This difficulty can be overcome in part either by the use of dilute solutions, by efficient washing with cold solvent, or by repeating the crystallization.

The crystallization procedure may be applied advantageously to the mixed fatty acids of most fats and oils without preliminary treatment. Thus, in most cases, the acids may be divided into three or four solubility groups: saturated, monoenoic, dienoic, and trienoic. In the case of the fish oils and the lipids of certain animal organs, such as the adrenal gland, the trienoic acids may be replaced by other highly unsaturated acids of the four, five, and six double bond types. In this very general classification each solubility group may include one or more minor component acids. With fats and oils of more complicated fatty acid composition, the method may be more effectively applied to individual carbon series of acids, thereby necessitating the preparation and efficient distillation of the methyl esters. If desirable, the esters themselves may be crystallized from solvents.

III. FRACTIONAL CRYSTALLIZATION OF THE FATS AND OILS

Before 1936 fractional crystallization was applied to the study of the fats and oils primarily as a method for the isolation of individual, naturally occurring glycerides. Reviews of this work have been given by Lewkowitsch (91), Grün (52), Schönfeld and Hefter (107), and Hilditch (61). Of these, the review by Hilditch (in reference 107) is the most complete. Furthermore, in this review Hilditch discusses two allied lines of investigation,—namely, the crystallization of the hydrogenated fats and the crystallization of the brominated fats and oils, both of which are not within the scope of the present paper. Since 1936 Hilditch and his coworkers have published a series of papers in which crystallization has been used as a preliminary step to the application of their chemical methods of study of fat structure. Because of the relatively low solubilities of the glycerides, and because the fats crystallize easily from solvents, most of the work so far reported has employed temperatures above 0°C.; hence it will be reviewed only briefly here. Only very recently have low temperatures been applied to the crystallization of the lower melting constituents of oils.

A. The isolation of naturally occurring glycerides by the fractional crystallization of fats

The isolation of naturally occurring glycerides by fractional crystallization, probably attempted before 1820 by Chevreul, was reported in detail by Duffy (42) in 1853. By a series of thirty-two crystallizations of mutton tallow from ether at 16°C., he succeeded in isolating 8 g. of tristearin from 2 kg. of the tallow. A similar experiment with beef tallow yielded only 2 g. of the same glyceride. Incidentally, he described double melting point phenomena which he called the "isomeric transformation of fats." Heintz (56) also used ether as a solvent in 1855. Blyth and Robertson (12) in 1889 crystallized butterfat from alcohol-ether and obtained the mixed triglyceride butyroöleopalmitin. When ether has been used as a solvent, the glycerides have been caused to crystallize by evaporation of the solvent or by the addition of alcohol, in which they are sparingly soluble. Other solvents which have been used are chloroform and ether by Fritzweiler (51), acetone by Klimont (81), and chloroform and acetone by Seitter (110).

Since about 1900 most of the researches on crystallization of fats have been reported by Klimont (80-87), Amberger (1-5), and Bömer (13, 16-21, 23-25), and their coworkers. Other important investigations, however, have been described by Heise (57), Henriques and Künne (59), Holde and Stange (75), Hansen (53), Kreis and Hafner (89), Fritzweiler (51), Seidenberg (109), Brash (27), Hilditch and Jones (64), and Kino (79). In Hilditch's review of this work (107), some twenty-five glycerides are

described as a result of the application of this technic. Among these are the simple triglycerides of lauric, myristic, palmitic, stearic, and oleic acids. The remainder are mixed triglycerides. About twenty fats and oils have been thus studied. In some cases literally hundreds of crystallizations were necessary in order to achieve the isolation of pure compounds.

The general procedure, used by Bömer and typical of the method (20, 24), is briefly described as follows: 1 to 2 kg. of the fat is dissolved in 2 to 3 volumes of ether, benzene, chloroform, or a similar fat solvent. The solution is allowed to stand at progressively lower temperatures for 1 to 24 hr., or the solubility is lowered by the addition of alcohol, and the resultant crystal fractions are removed by suction filtration. Each of the fractions is further divided by similar procedures into three or four subfractions, finally combining those that melt within a 5°C. range. Unsaturated glycerides are removed by saturating them with the Wijs reagent and crystallizing until they are free of halogen. As the refractionation proceeds, subfractions are united of 2°, 1°, and finally 0.5°C. melting range. Crystallizations are continued until the desired results are obtained.

Bömer and Limprich (22) have reported an ingenious crystallization method for the identification of certain glycerides, and Bömer (14, 15) has employed the technic for the detection of tallow in lard and of lard in vegetable fat and butterfat.

As a method of study of the composition of fats the method of crystallization has been disappointing, mainly on account of the complexity of composition of the naturally occurring glyceride mixtures.⁴ Even with a fat containing only three fatty acids, and few so simple as this are known, there are theoretically possible eighteen triglycerides: three homogeneous simple triglycerides, twelve partly homogeneous glycerides, such as palmito-distearin, and three completely heterogeneous glycerides, such as palmito-stearomyristin. It is clear that if all three of the fatty acids were saturated and of high molecular weight, the differences in solubility would be too slight to make practical separations possible. Even if one of the acids were unsaturated, such as oleic, if anything approaching the theoretical number of possible compounds were present, they would display such a gradually changing series of solubilities as to make separations very difficult.

The value of the crystallization technic as applied to the study of fat structure is excellently summarized by Hilditch (60) in the following tribute to Bömer and to the method, as follows: Bömer "subjected many fats to examination by the physical method of crystallization, supplemented in many cases by vacuum distillation. His methodical and sustained attack on this problem, involving several hundred crystallizations in the case of

⁴ The complexity of the composition of fats and oils is further discussed in the paper by Dr. Longenecker (Chem. Rev. 29, 201 (1941)) in this Symposium.

cocoanut and palm kernel oils, demonstrated beyond all doubt the mixed nature of naturally occurring triglycerides. The complexity of the mixtures of glycerides usually present and the close similarity in solubility and other physical properties made it impossible, as we now realize, to achieve adequate separation of most fats by physical methods alone and Bömer was rarely able to obtain more than fragmentary quantitative information; and when in due course the use of chemical methods of attack gave quantitative proof of this tendency for the different acyl groups to be distributed very evenly or indiscriminately amongst the glyceride molecules, the validity of Bömer's results was amply corroborated."

B. The separation of fats into crude fractions, preliminary to further chemical study

In 1936 Hilditch and coworkers (38, 39, 62, 63, 65-74) began to employ crystallization, usually from acetone at 0°C., as a method of dividing fats into a few simple fractions as a preliminary step before applying the chemical method to the study of the glycerides. Such fractions were intended to contain a relatively simple mixture of glycerides of similar solubilities. The number of fractions varied from two to seven, depending on the fat being studied. For example, Bushell and Hilditch (38) separated Borneo tallow into six fractions of iodine numbers 29.6, 33.9, 33.5, 33.3, 48.3, and 47.2. Likewise from cacao butter Hilditch and Stainsby (74) obtained five fraction of iodine numbers from 28.2 to 50.1.

The application of very low temperatures to the crystallization of an oil was reported in 1901 by Holde and Stange (75), who cooled an ether solution of olive oil to -40 to -45°C. The crystal fractions were recrystallized at 0°C., whereby an impure oleodipalmitin was obtained. They demonstrated for the first time the almost complete absence of triolein in this oil.

Within the past year two low-temperature crystallizations of cottonseed oil have been reported. Hilditch and Maddison (66) subjected a 33 per cent solution of the oil in acetone to a temperature of -10°C. The final crystallization of the liquid glycerides was from a 20 per cent solution at -35°C. The iodine numbers of the six fractions finally obtained were 38.3, 57.0, 97.0, 107.9, 124.7, and 134. Obviously this represents a wide range of unsaturation in the several fractions.

The most detailed application of the method to any oil has been reported by Riemenschneider, Swift, and Sando (104). One kilogram of cottonseed oil was crystallized according to the scheme in chart 1. Data on the several fractions are shown in table 5.

The separations achieved in this work are remarkable, especially fractions F and G which contained, respectively, 71.4 and 79.0 per cent of linoleic acid. Although these fractions represent only 19 per cent of the

original oil, they undoubtedly contain considerable amounts of trilinolein, thus demonstrating the occurrence of small amounts of this triglyceride

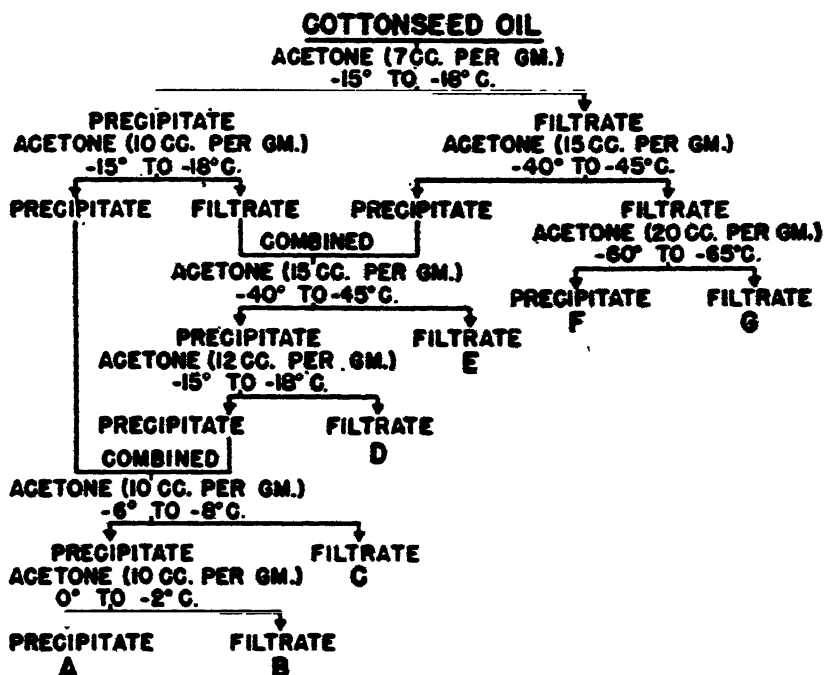


CHART 1

TABLE 5

Analysis of fractions obtained by crystallization of cottonseed oil

FRACTION	WEIGHT	IODINE NO. (WLS)	THIOCY- ANOGEN NO. (3 HR.)	FATTY ACID COMPOSITION		
				Linoleic acid	Oleic acid	Saturated acids
	grams			per cent	per cent	per cent
A.....	149	52.5	30.8	25.0	10.6	64.4
B.....	11	58.2	33.5	28.5	10.2	61.3
C.....	18	66.3	38.3	32.3	12.0	55.7
D.....	451	107.1	62.1	51.9	19.9	28.2
E.....	154	133.4	74.8	67.6	18.8	13.6
F.....	145	140.6	78.8	71.4	19.7	8.9
G.....	48	148.3	79.9	79.0	13.3	7.7

in cottonseed oil. In comparison with the above results, it is interesting to note that the same oil, when subjected to molecular distillation, gave fifteen fractions, the linoleic acid content of which ranged from 48.0 to

59.6 per cent. The advantages of fractional crystallization in this instance are obvious.

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THE POLYMORPHIC FORMS OR PHASES OF TRIGLYCERIDE FATS¹

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I. INTRODUCTION

The remarkable way in which fats melt, resolidify, and then melt again on further heating has been the subject of experimentation and speculation since the time of Chevreul. The double melting of tristearin was one of the first examples to receive extensive study, and Guth (17), among others, found that when the molten triglyceride was quickly chilled, it thereupon melted at 55°C., but shortly resolidified, to melt again at 71°C. as the heating proceeded. The cause for this behavior was usually assigned, mainly on the basis of analogy and assumption, to the existence of two

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different isomeric modifications, one melting at the lower and one at the higher temperature.

The study of the polymorphism of the triglycerides originated in such melting-point phenomena, but over the years, the description of the melting behavior of tristearin (or of other glycerides) has seldom been clearly given, and indeed, as will appear below, not until the advent of Malkin's (6, 7, 29, 30, 31) work in the last decade has any definite and clear basis for an understanding of the melting behavior of fats been available in the literature. To be sure, the predecessors of Malkin in the study of pure triglycerides had laid the foundation, and the many researches on simpler long-chain compounds, such as the fatty acids, had indicated the plan of attack, but it is notable that much of the more stimulating earlier work was performed, not upon single pure chemical compounds, but upon the complex mixtures of glycerides in the natural fats. Many of the concepts of Van Roon (48) and others on the fats of commerce, while not clear as to the nature of the crystalline structures involved, may be applied with profit to polymorphic and phase studies of triglycerides.

The point of view of the present paper is the consideration of triglycerides in terms of the solid physical forms or phases in which they exist. Emphasis has been and will be placed on melting and solidification phenomena, because historically the study of the polymorphic forms has been based almost exclusively on observations intimately associated with the heating and cooling of fats. Not until recently have other phenomena and other methods been brought to bear on the problems concerned with the phase relations of the triglycerides. Even these later investigations have invariably been supplemented and substantiated by studies of melting and crystallization behavior.

II. THE MEANING OF THE MELTING POINTS OF FATS

In the realm of commercial fats it has been appreciated for many years that the melting point of a sample depended on (1) the particular melting-point method used, and (2) the previous history of the sample. The typical condition of affairs has been indicated in the admirable statement of Van Roon (48) with regard to cacao butter: "Many methods have been proposed for determining the melting point of cacao butter, and in each the melting point is defined differently. None of these can be called generally useful. They simply lead to confusion, which is increased by the fact that the treatment of the fat in preparation for the melting point determination has a great effect on the result."

Unfortunately, such recognition of the difficulties has not always prevailed in the field of glyceride chemistry, and all too often melting-point data for glycerides are reported, which are not susceptible to exact com-

parison, even assuming the glycerides in question are of identical composition. For example, the melting point for β -laurodistearin has been given by various workers as 36° , 52° , 56.5° , 58° , 59.8° , 60.5° , and 68.5°C .

Until the stability and equilibrium relations between the various forms or phases in which a given glyceride may occur are clearly understood, such a profusion of melting-point results makes it practically impossible to establish anything precise in the way of characterizing properties. Each glyceride must be considered as a one-component system capable of changing form and properties without change in composition.

Since the work of Othmer (38), Loskit (27), Joglekar and Watson (22), and especially the later contributions of Malkin (6, 29, 30, 31) have made it clear that triglycerides can exist in at least four different modifications,—the γ -, the α -, the β' -, and the β -phases,—it is necessary to consider the actual melting-point determination in terms of these phases. Each phase has its own melting point, and in considering the melting point of any triglyceride it is necessary to realize which particular form of the glyceride is involved.

Perhaps the most significant method of ascertaining the melting point of an already solidified or processed fat is the so-called "quick method", essentially that described by Timmermans (47). The sample, without previous liquefaction, is placed in a small, thin-walled capillary, and this is thrust into a bath of known temperature. If the sample melts, similar samples are then thrust into the bath at lower temperatures, until a temperature is discovered at which the sample barely melts.

Suitably applied, this method also serves to distinguish the various phases mentioned above (30) from one another. Thus a very small quantity of a molten fat may be drawn into a thin-walled capillary, then quickly chilled. Under proper chilling conditions the γ -phase of the triglyceride is produced, but it is unstable and its melting point therefore must be obtained, not by slowly raising the temperature of the bath, which would allow it to be converted into a more stable phase, but by thrusting the capillary quickly into a bath of known temperature. At the gamma melting point liquefaction ordinarily occurs, followed by resolidification into the α -, β' -, or β -modifications, depending on conditions. The melting points of these phases then must be determined by similar methods, in which, of course, the production of the correct phase prior to the determination is imperative.

Even the special melting-point methods may fail to yield a reliable figure with some compositions, and cooling-curve and other techniques are required to obtain the temperature of melting (7).

From what has been said, it is apparent that the legendary conception of "double melting" of fats (see, especially, 42) must be replaced by "multiple

melting", and the melting points must be given in terms of the different physical forms in which the solid fat may occur.

III. THE DEVELOPMENT OF THE CONCEPT OF POLYMORPHIC FORMS IN FATS

The initial development of the concept of polymorphic forms in triglyceride fats goes back to the obscure beginnings of organic chemistry. It is remarkable that, as early as 1853, Duffy (11), building on the experience of earlier observers, actually discovered three melting points for "stearin" from mutton fat many times recrystallized. These three melting points agree rather well with the three melting points obtained for tristearin by the most reliable modern observers. Duffy noted that, with considerable impurity present, it was difficult to obtain the highest melting points, and that, as impurity was eliminated, it was less and less easy to find indications of the intermediate melting point. He stated clearly, apparently for the first time, (1) that the solidification temperature and the lowest melting point were practically identical and (2) that the melting point of crystals obtained from solvents was identical with the highest melting point obtainable with the glyceride itself. The assumption was made that the melting behavior arose from some form of isomerism.

It appears that many later observers, in the course of years of quoting and re quoting, lost sight of the best of Duffy's work. They still, for the most part, kept to the idea of isomerism as the cause of double melting. The intermediate melting point was missed, probably because of the increased purity of the tristearin employed in experimentation.

In 1902, Guth (18) determined the melting point of tristearin as 71.5°C. Moreover, this was described as the sole melting point, provided the sample was held in the capillary sufficiently long before the determination was made. If examined soon after placing in the capillary the fat appeared to undergo two distinct meltings, the first occurring at a much lower temperature than the other. Guth also made the very illuminating disclosure that, at the lowest observed melting point, complete melting did not occur unless a very narrow capillary and a very small quantity of fat were used. This is still good technique today and is necessary because of the rapidity with which the low-melting unstable γ -form is transformed, or the melt crystallizes to give the alpha or more stable phases.

As a result of his investigations, Guth concluded that the rapidly cooled sample, when it resolidified, had in reality not passed into the crystalline state, but was in a condition analogous to that of undercooled water or a supersaturated solution, which upon slight disturbance thereupon passed into a crystallized condition. These ideas are not so far removed from Malkin's (7) modern conception of the lowest melting triglyceride as a "glass".

Further contribution to the relationship of the materials melting at the two temperatures was made by Grün and Schacht (15). They prepared three mixed triglycerides, each of which exhibited an unstable (low-melting) and a stable (high-melting) form. It was then determined that the unstable form was converted into the stable modification when inoculated with the latter. The conclusion was reached, valid today, that the change was monotropic, no reversions occurring under any conditions. These investigators, however, still labored under the misconception that isomerism was involved and, indeed, Custodis (8) found later, probably erroneously, that the unstable form gave only half the theoretical value in the freezing-point determination of molecular weight.

Le Chatelier and Cavaignac (26) now introduced evidence to show that the melting of a triglyceride was a reversible process, the peculiar behavior reported by earlier workers being due to the slow velocity of the melting or crystallization. If the melting or solidification was carried out with an extreme degree of slowness, the reverse change occurred within 0.1 to 0.2°C. Rapid determinations were accordingly designated as the cause of divergent melting points. Up to this point they were perfectly correct as to the physical evidence, but no fact was found necessitating the assumption of the existence of different polymorphic forms, although such forms were not excluded. The issue was confused by minimizing the long-substantiated fact that solidified tristearin could be made to melt and resolidify at as low a temperature as 55°C., although the very same sample would yield a reproducible, reversible high melting point of about 71°C. However, of great importance was the establishment of the reversibility of the upper melting point.

In 1907 Bömer (5), substantiating Duffy (11), showed definitely that a high-melting modification always resulted when triglycerides were crystallized from solvents, and felt therefore that the melting point of such a preparation was to be regarded as the true melting point. He also indicated more definitely than most the exact conditions for obtaining the low melting point.

The unstable, glass-like nature of the low-melting modification was from time to time emphasized, other workers looking upon it as an anisotropic liquid phase (21). Malkin (7), the latest large contributor to the study of the polymorphism of triglycerides, regards the γ -form as a "glass".

The first definite intimation of a rediscovery of still other modifications than the so-called stable and unstable forms came in 1915, when Othmer (38) listed not two, but three, melting points for trimyristin and tristearin. Othmer's conceptions seemed to involve simple crystalline polymorphism and not isomerism, and thus in some respects a clarification of the problem resulted.

Loskit (27) a little later contributed strong confirmatory evidence for at least three different forms in which tristearin and other triglycerides could exist under proper conditions. Moreover, the microscopic structure of the three forms was specified,—the low-melting phase being of a “colloidal” glassy nature, the intermediate form consisting of spherulitic crystals, and the stable form separating in crystalline needles.

Efremov (12), in 1927, for all the obvious lack of purity of his materials and inaccuracies in his numerical values, obtained definite evidence of the three polymorphic forms in tristearin and tripalmitin and, as related below, made confirmatory experiments in binary glyceride systems.

Apparently ignoring the purport of Othmer's, Loskit's, and Efremov's experiments, and reviving ideas connected with isomerism, Joglekar and Watson (22), as late as 1930, concluded that only two different solid modifications of triglycerides existed. The stable form, which they call alpha (this is the beta form of Malkin, whose nomenclature is employed throughout this paper) separates in crystalline condition from solvents, and the unstable variety occurs when the molten fat is chilled, thus far confirming many previous investigations. In spite of its limitations, the paper of Joglekar and Watson contains much that is fundamental, such as an unusually clear exposition of the methods and conditions required to obtain the stable and unstable forms, and what appear to be highly accurate melting points. These investigators seem to have been the first also to note clearly in triglycerides the phenomenon of transformation in the solid state, so often seen in commercial fats. Thus, after chilling a sample of molten tristearin it was found that “the solid melted at 55°, the melting point of the β form (Malkin's γ), but resolidified in a few moments, showing that numerous nuclei of the α form (Malkin's β) had been produced by chilling. This formation of nuclei is, no doubt, responsible for the change of the β (Malkin's γ) into the α (Malkin's β) form in the solid state at room temperature frequently observed during the experiments. When it takes place there is a remarkable swelling into sponge-like formations, and the containing vessel is often broken”.

While Joglekar and Watson (22) and later Rao and Jatkar (39) recognized but two modifications in simple glycerides, it will be recalled that Duffy, Othmer, Loskit, and Efremov believed that three forms were possible. Furthermore, in 1932, on the basis of melting-point and microscopic experiments, Weygand and Grüntzig (49, 50) came to the conclusion that as many as seven different polymorphic forms were able to exist. Their work seems to have been carried out with considerable accuracy, although it would appear that their original results in reality indicate with certainty only three forms for tristearin.

It is clear, therefore, that a very considerable body of evidence had been

built up pointing to the occurrence of several distinct glyceride phases, yet until recently there had been no adequate description or characterization of these forms and no clear and fully convincing evidence as to their number for any particular glyceride.

IV. THE POLYMORPHIC FORMS OF SIMPLE TRIGLYCERIDES

A. INTRODUCTION

Before proceeding to the elucidation of the polymorphism of the simple triglycerides, there should be a brief consideration of the background of information on the polymorphism of the simpler long-chain compounds related to the triglycerides. There is a store of information here to draw upon, from which the following points are pertinent and significant in relating the larger field of long-chain compounds to that of the triglyceride fats.

(1) Polymorphism is almost universal in long-chain compounds.

(2) Long-chain compounds crystallize generally with two, four, or eight molecules in the unit cell.

(3) There are two main types of polymorphic forms, for which a conventional nomenclature has been fairly widely accepted: (a) Alpha form: In this form the long chains crystallize in positions perpendicular to the planes determined by the end groups. This form usually exhibits no alternation² in physical properties for odd and even number of carbon atoms. Only one alpha form exists for a given compound. (b) Beta forms: In these, the long chain crystallizes at an angle, which is not a right angle, to the plane of the end groups.³ These forms generally exhibit alternation in physical properties between odd and even series. More than one such form may exist for a given compound.

(4) Transformations from one form to another are slowed up when impurity is present. Impurities may be other members of the same homologous series.

(5) Compound formation may occur between near members of a homologous series (44).

(6) Not only does the second component of a binary system tend to slow up transformation from one form to another, but sufficient amount of the second component may cause a form which is unstable in the single-component system to become the truly stable form in the binary system (45).

² For a discussion of the geometrical basis of alternation, see Malkin (23). For a critical discussion of this geometrical explanation, see Eyring (13).

³ For a presentation of the possible interrelation of the alpha and the various beta forms, see Schoon (43) and Fuller (14).

B. SIMPLE TRIGLYCERIDES

The long-anticipated phase basis for systematizing and explaining the behavior of triglycerides became a definite reality in 1934, when Clarkson and Malkin (7) presented convincing x-ray and other evidence on the polymorphism of triglycerides of chain length from ten to eighteen carbon atoms.

It now appears certain that the simple triglycerides occur in at least three phases,—the gamma, the alpha, and the beta. (A fourth form intermediate in stability between the alpha and beta forms occurs for the triglycerides with an odd number of carbon atoms.) The melting, solidification, and other properties of the triglycerides must therefore be interpreted and studied with respect to the phase or phases present.

Insofar as they can be applied generally, the main identifying characteristics of the three polymorphic forms are listed in table 1.

1. Phase melting points

When the melting points of the three modifications are determined for the simple glycerides, it is found that the beta phase exhibits the familiar property of alternation, whereas the alpha and gamma forms do not (7).

It is evident from figure 1 that tristearin, for example, may give a melting point of 71.5°, 65°, or 54.5°C., depending on which phase is present. As a practical matter, a given sample of tristearin may yield almost any melting point over this range, depending on the history of the sample before the determination is made. Accordingly, the special meaning of melting point, outlined earlier, must be recalled in dealing with the melting of the three triglyceride phases. The determination of melting point includes the processing necessary to induce the formation of the particular phase in question. Thus, to obtain the gamma melting points shown in the lower curve of figure 1, the triglyceride in molten condition should be rapidly chilled in a narrow, thin-walled capillary. Immediately the chilled sample is thrust into a bath of known temperature to see whether or not melting occurs. In either case a well-melted and newly chilled sample is thrust into a bath at higher or lower temperature, and so on until the true melting temperature is obtained.

By this method the melting point of the γ -form of the triglyceride is obtained. Properly determined, it is a definite, characterizing property.

A major concern in determining the true gamma melting point is obtaining the correct chilling rate to induce the formation of the gamma form and it alone. While the glycerides of C₁₃, C₁₄, and C₁₅ acids solidify in the gamma form when chilled in an ice-salt mixture, members higher in the series solidify readily in ice. Tristearin, in fact, solidifies in the gamma form if merely cooled to room temperature.

TABLE 1
The polymorphic forms of simple triglycerides (tristearin)

	FORM		
	Gamma	Alpha	Beta
Relative stability.....	Least	Intermediate	Most; the only thermodynamically stable form
Melting point.....	Lowest; non-alternating for odd and even number of carbon atoms	Intermediate; non-alternating	Highest; alternating
Gross appearance.....	Vitreous, translucent	Intermediate	Generally opaque, even powdery
Microscopic appearance..	Tiny black and white uniaxial crosses; a mosaic between crossed nicols	Much larger uniaxial crosses, not as brilliant	Small parallelograms; best, no nicols (can form spherulites)
X-ray evidence: Short spacings*.....	One, diffuse, 4.2 Å.	One, fairly sharp, 4.2 Å.	Multiple, sharp; 3.7, 3.9, 4.6 Å. strong; 5.3 Å. weak
Long spacings†.....	None found	Longer; two chain lengths (perpendicular chains)	Shorter; more than one, less than two, chain lengths (tilted chains)
Crystal structure.....	Possibly glass or liquid crystalline	Possibly orthorhombic (mesomorphic?)	Monoclinic
Density.....	Least	Intermediate	Greatest
Heat of crystallization...	44 cal. per gram	Undetermined; probably intermediate	62 cal. per gram
Dielectric constant.....	Near liquid value	Undetermined	Lowest
How obtained:			
From liquid.....	Rapid cooling	Slow cooling	Very slow cooling
From other solid.....	Not possible	From γ -form near gamma melting point	From α -form near alpha melting point
Existence of type form in other long-chain compounds.....	Apparently <i>uniquely</i> in triglycerides	Quite general in mono- and diglycerides, paraffins, and esters; <i>not in saturated fatty acids</i>	Quite general in mono- and diglycerides, paraffins, esters, <i>and fatty acids</i>

* Short spacings refer to distances of separation of hydrocarbon chains.

† Long spacings refer to lengths of hydrocarbon chain.

It must be emphasized that the size of the sample is important. A large sample, cooling at unequal rates from surface to center, will, in most examples, exhibit a mixture of phases. Hence for melting-point determinations the necessity of the restriction to small quantities in thin-walled capillaries is apparent.

Usually the melting point of the alpha or intermediate phase is not so readily ascertained, since this form is probably never obtained in an absolutely pure state. This is because the melt may crystallize in a mixture of forms, or the gamma phase may transform to both alpha and beta, and the alpha, especially in some glycerides, may transform rapidly to beta.

In general, however, the melting point of the alpha phase is detected (7) by first obtaining the melting point of the gamma phase in a bath at a temperature where it just melts and resolidifies: $\text{gamma} \rightarrow \text{liquid} \rightarrow \text{alpha}$.

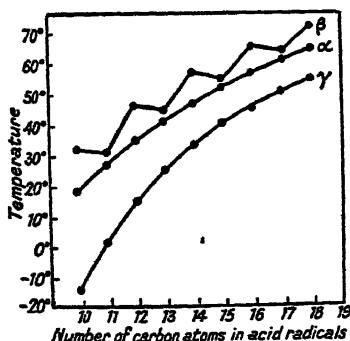


FIG. 1. Melting points of triglycerides

The melting point of the alpha form is then found by thrusting the capillary into a bath at the correct melting temperature as indicated by trial. Here again large samples obscure the result, since the α -phase changes to the β -phase in the solid state without melting, i.e., below the true alpha melting point. Hence it is that the usual melting-point procedure, wherein the temperature of the sample is raised gradually, never discloses the alpha melting point, although it may sometimes give an indication of the gamma melting point.

The maximum melting point, that of the stable β -phase, may be determined readily in many fats by ordinary procedure. With others, it is necessary that the β -phase be present initially. This condition is not always easily attained, since impurity or special composition may lead to an extremely slow formation of the β -phase in the stabilized fat. In such cases a very slow rise in the temperature of the bath, with periods of

holding at temperatures below the melting point (tempering), are required to develop the true maximum melting temperature of the sample. For such samples the dilatometric curves illustrated by Hofgaard (20) yield illuminating results, and in some instances cooling and heating curves enable the true melting point to be obtained (30).

For some of the simple triglycerides table 2 lists the melting points of the three phases as determined by different workers. Considering possible differences in purity of samples and in technique, the values for a given phase are, in most instances, remarkably close, and amply support the existence of three distinct modifications.

While Clarkson and Malkin regard their values for the gamma melting point as being ranges of temperature, rather than points, it is probable that more careful technique would define these with considerable sharpness.

The whole discussion has been based on the assumption that but three polymorphic forms exist in the simple triglycerides. It will be recalled that Weygand and Grüntzig (*loc. cit.*) have reported seven forms for the even-carbon chain and four for the odd. Their results were based on melting-point and meticulous microscopic studies. Malkin in his first paper on triglycerides has seemed to dispose fairly of their case with regard to the triglycerides with an even number of carbon atoms and, indeed, inspection of the results as reported in table 2 will show how closely the melting-point figures fall in three well-defined groups.

However, Grüntzig (16) still maintains his earlier stand and reports a private communication from Malkin accepting the existence of four forms in the odd triglycerides, in confirmation of Weygand and Grüntzig's findings. It will be noted later that Malkin has found four forms for practically all mixed triglycerides that he has studied.

On the basis of the best available evidence it may be assumed for the present that in the simpler triglycerides the more important even members exhibit but three forms, while the odd members exhibit four.

Melting-point information on the simple unsaturated triglycerides is necessarily meager, considering how recently pure materials have become available and how low melting the compounds are. The data of Wheeler, Riemenschneider, and Sando (51) on triolein, combined with those of Carter and Malkin (33) for trierucin, show that the unsaturated homologous series of which these are members follows the saturated series in polymorphic behavior, each member exhibiting three melting points. Trilinolein, on the other hand, apparently exhibits but two polymorphic forms. The unsaturated glycerides show more rapid transformation of forms than do the corresponding saturated compounds.

The isomeric trielaidin and tribrassinidin also fit into the general scheme with three polymorphic forms, according to Carter and Malkin (6).

2. Cooling and heating curves

With the aid of cooling and heating curves, judiciously performed, a valuable insight has been obtained into the phase transformations which take place during the crystallization and melting of fats. This insight is deeper and more complete than can be obtained with melting points alone.

Malkin (7) was not the first to employ cooling and heating curves, although his curves are related more definitely to the phase changes which occur. Nicolet (37) revealed valuable indications as to the polymorphic behavior of tristearin and closely related commercial fats, as a replotting of his data will show (see figure 2).

While not explicitly stated in his paper, it is clear from the curves, especially in the light of other more recent information on the polymorphism

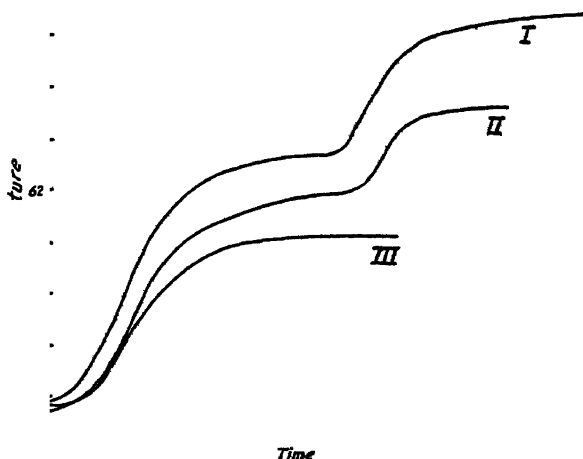


FIG. 2. Nicolet's cooling curves for triglycerides. Curve I, tristearin; curve II, hydrogenated linseed oil; curve III, hydrogenated soybean oil.

of triglycerides, that his sample of tristearin supercooled to a minimum approximating the gamma melting point. Solidification in the gamma form then occurred, followed by transformation and then crystallization in the alpha form with a rise in temperature, a flat in the curve roughly approximating the alpha melting point. Further transformation and crystallization in the beta form then took place with another temperature rise to a maximum, which is approximately the temperature of melting of the beta phase. This behavior refers specifically to tristearin, but an inspection of the curve for hydrogenated linseed oil (iodine value = 2.7; 7.5 per cent non-stearic acids) indicates a very similar sequence of events during the cooling of this fat. It is important to observe also that the corresponding curve for hydrogenated soybean oil, which contains 8.1

per cent of non-stearic acids and is thus slightly further removed from tristearin in composition, shows no second rise, although (according to Nicolet) such a rise did occur when the sample was seeded. A hydrogenated cottonseed oil (28 per cent palmitic acid), departing considerably in composition from tristearin, exhibited no second rise even with seeding. This behavior emphasizes the marked retarding influence of admixture on phase transformation, an influence the potency of which must be constantly kept in mind when dealing with commercial fats.

Still more pertinent and revealing interpretations are derived from Malkin's cooling and heating curves (see figure 3) for various triglycerides. His work was performed with small amounts (1 g.) of material in a jacketed system; temperatures were followed by means of thermocouples. The dotted cooling curve (for tristearin) shows the results of rather rapid cooling. It has one nick corresponding to the gamma melting point, again

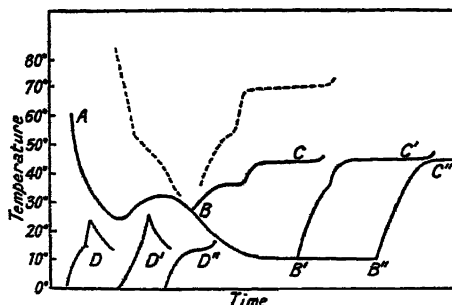


FIG. 3. Malkin's cooling and heating curves for various triglycerides

showing the gamma melting point as the approximate limit of supercooling.

The dotted heating curve for tristearin shows by the first deflection a partial melting at the gamma melting point, then a sharp rise indicating alpha and beta crystallization, probably accompanied by alpha-to-beta transformation. There is no evidence of the alpha melting point, owing to the relatively slow heating gradient. Finally the long flat indicates beta melting.

Consideration of the solid curve (for trilaurin), marked $ABB'B''$, shows that, under the particular experimental conditions, the trilaurin did not supercool to the gamma melting point ($15^{\circ}\text{C}.$) as it would with a faster cooling rate. The fat supercooled to about $25^{\circ}\text{C}.$ to crystallize in the alpha form with a rise, due to heat of crystallization, almost to the alpha melting point.

The heating curve BC shows two distinct arrests. The curve starts above the gamma melting point, so the first arrest indicates melting of

alpha to liquid, probably accompanied by transformation $\alpha \rightarrow \beta$. Upon the disappearance of the alpha form, the crystallization of the beta form raised the temperature sharply to the second arrest, corresponding to the melting point of the beta phase.

The conversion of one form to another with elimination of an arrest in the curve is indicated in $B'C'$ and $B''C''$. The samples for these latter curves were held at room temperature (above the gamma melting point) for 1 and 3 hr., respectively. (Note that again no indication of the gamma form appears, although the curve starts below the gamma melting point. The trilaurin had crystallized entirely in other forms above the gamma melting point.) The result of storage at room temperature was that the alpha arrest disappeared, showing that, by tempering, the alpha form had been converted to the stable beta form.

The curves D , D' , and D'' for trilaurin are examples of Malkin's heating-curve method of determining the melting point of the gamma form. The molten sample was rapidly chilled in a mixture of dry ice and ether. It was then set in a bath which had been found by trial to be near the gamma melting point. For the case when the temperature was too high, the curve rose rapidly past the temperature of the bath without exhibiting any pronounced break (D'). If the temperature was too low, there occurred a pronounced break in the curve before it began to rise sharply (D''). The gamma melting point was taken as the intermediate temperature at which the arrest was just sharply noticeable (D).

Since the quantity of fat, the cooling and heating gradient, and the special nature of the particular glyceride all affect the cooling and heating curves, it is not to be expected that the arrests should correspond under all conditions to the exact melting points or that arrests corresponding to every polymorphic form of the particular glyceride should be obtainable under conditions which can be prescribed as general for all glycerides.

In obtaining cooling curves, if the cooling rate is too fast, manifestations of the higher melting forms are apt to be lacking. If the rate is too slow, supercooling may be limited and manifestations of the unstable forms may not be obtained. Similarly for heating curves, too fast a rate may fail to reveal the more stable, higher melting forms. Too slow a rate may obscure the unstable forms. In any case, but more especially in the heating curve, the course of the physical changes depends on the previous history of the starting material, i.e., in the case of solid material for a heating curve the course of the curve depends in large part on the phases already present, as determined by previous crystallization history.

The same, or what seems to be corresponding, treatment for different triglycerides will not always yield the same manifestations of polymorphism. The greater spread of melting point and the greater transformation

rates of lower members of the homologous series make each material practically a special case. A further complication is the slower transformation rate of glycerides with chains of odd carbon number as compared with the rate for even-number glycerides.

As a general rule, modified by the above considerations, it appears that extremely slow cooling from initial temperatures at a little above the beta melting point causes beta crystallization. From higher temperatures and with somewhat quicker cooling the alpha phase is obtained. Rapid chilling to a temperature below the gamma melting point induces the formation of the gamma modification. Of the two forms, alpha and beta, the former separates first, provided the liquid is held below the alpha melting point. Upon raising the temperature of a cooled sample, transformation to the more stable forms may occur, and variations in cooling and tempering conditions lead to mixtures of phases, which may sometimes be taken for additional phases (7).

3. Microscopic evidence

Information on the microscopic structure of fats is widely scattered, but for the most part does not take into account the different modifications. The most pertinent and valuable contributions with respect to triglyceride polymorphism are found in the work of Loskit (27). Additional material is given by Weygand and Grüntzig (49, 50) and by Malkin (30). No completely satisfactory characterization of the various phases by microscopic methods has been brought forward, but the following descriptions represent the best available information:

Gamma phase: Between crossed nicols there usually appear very tiny uniaxial crosses, indicating spherulite formation. The whole field may appear as a mosaic of tiny points of light so small as to make the detection of the uniaxial crosses difficult (27, 40, 49, 50).

Alpha phase: Much larger uniaxial crosses are formed, distinguished from those of the gamma phase by size and by the lesser contrast between the dark and light portions of the field. Conversion to the beta form is readily observed by destruction of the pattern (Malkin).

Beta phase: Spherulites are formed but not with such a striking pattern of uniaxial crosses. The form appears as a multitude of usually perfect little parallelograms of various sizes and shapes, some almost needle-like, others broader. Such shapes are also obtained by crystallization from organic solvents. The crystals are small for the higher members of the homologous series and larger for the lower members.

4. X-ray diffraction patterns

Perhaps the most conclusive differentiation of triglyceride phases comes from x-ray diffraction patterns. Not only does the pattern designate the

phase present, but, of equal value, it enables changes and transformations to be followed. Thus, for example, the diffraction pattern indicates the conversion of the gamma form to alpha and beta as a sample containing the unstable modification is aged at a given temperature. If a characteristic ring pattern can be recognized as pertaining to one definite phase, then nothing need be known, as to calculated spacings or unit cells, in order to follow the subsequent career of the phase.

As pointed out by Davey (9), the quantitative x-ray study of mixtures of phases can be carried on accurately only if mixtures of known phase composition are used as a basis. For the fats no published x-ray evidence

TABLE 3
X-ray spacings for phases of triglycerides

GLYCERIDE	LONG SPACINGS		BETA SHORT SPACINGS	
	Beta	Alpha	Even	Odd
Tristearin.....	45	50.6	3.7, 3.9, 4.6, 5.3	
Trimargarin.....	43.5	48.5		3.65, 4.0, 4.6, 5.3
Tripalmitin.....	40.6	45.6	3.7, 3.9, 4.6, 5.3	
Tripentadecylin.....	38.9	42.9		3.65, 4.0, 4.6, 5.3
Trimyristin.....	35.8	41.2	3.7, 3.9, 4.6, 5.3	
Tritridecylin.....	34.1	37.7		3.65, 4.0, 4.6, 5.3
Trilaurin.....	31.2	35.6	3.7, 3.9, 4.6, 5.3	
Triunidecylin.....	29.6	33.0		
Tricaprin.....	26.8		3.7, 3.9, 4.6, 5.3 4.2*	
Trierucin.....	51.1	55.0	4.2 diffuse† 3.70, 3.84, 4.03, 4.60, 5.24	
Triolein.....			Oil	
Tribrassidin.....	53.6	60.9	3.75, 3.95, 4.95, 5.35	
Trielaidin.....	44.1		3.75, 3.95, 4.95, 5.35	

* Alpha short spacings.

† Gamma short spacings.

is based on such technique, and accordingly the study of phase transformation has at best been qualitative. Another limitation in the use of the x-ray for fats has been the rapidity with which change in phase occurs under some conditions. Nevertheless, as applied by Malkin and his coworkers, the x-ray pattern provides a highly valuable means of attacking many of the problems connected with the polymorphism of the triglycerides.

For example, Clarkson and Malkin (7) found that a characteristic beta pattern is obtained when purified triglycerides are crystallized from solvents. If, however, the beta crystals are melted and then allowed to flow onto a thin glass strip, either the alpha or the gamma phase is obtained, depending on the rate of cooling, the pattern changing accordingly.

In the foregoing discussion the ring *pattern* characteristic of a phase has been emphasized, but in table 3 and figure 4 the actual measured long and side spacings for the various phases of triglycerides from tricaprin to tristearin are compared.

As has been said, it is not necessary to know the crystal spacings or the crystal structures derivable from them to study polymorphic changes, but to understand the nature of the polymorphic forms and their changes it is well to glean from x-ray information all that is there.

As Malkin makes clear, a full solution of the triglyceride structure must await such single-crystal studies as have been made by Müller (34, 35) on fatty acids and paraffins. However, from Malkin's x-ray data the following inferences may be drawn:

Gamma form: The hydrocarbon chains are probably in fairly general alinement in the micro-structure. However, according to Clarkson and Malkin (7), the single diffuse band in the x-ray spectrum indicates that

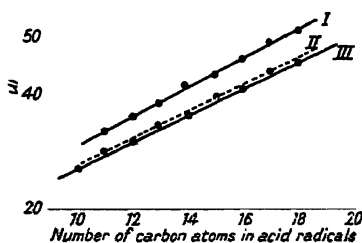


FIG. 4. Long x-ray spacings of triglycerides

the chains are "frozen" in random degrees of rotation about the chain axis. The diffuseness of the band indicates that distances between chains are not very precisely determined. The lack of long spacings, if it is not due to difficulty of observation, indicates that the ends of the hydrocarbon chains, i.e., the methyl groups and the glyceryl groups, do not determine planes.

Even liquids and glasses will give a diffuse band (for instance, near the melting point liquid paraffins give a spacing of 4.6 Å. (36), as compared with 4.2 for the gamma spacing), though possibly not so well defined as the gamma diffuse band. Clarkson and Malkin conclude that the gamma form is a glass. (It must be noted, however, that the gamma form is anisotropic, as previously mentioned; besides, it shows very considerable volume and heat changes on melting. The possibility that the gamma form is in a mesomorphic or liquid crystalline state does not seem to be ruled out.)

Alpha form: Here also the hydrocarbon chains are in alinement and

"frozen" in random degrees of rotation about the chain axis (Clarkson and Malkin (7)). However, the relative sharpness of the single line for side spacings indicates quite constant distances between hydrocarbon chains. The presence of long spacings proves that the ends of the hydrocarbon chains determine planes, and, further, the spacings are of such magnitude as to prove that the hydrocarbon chains are perpendicular to the end-group planes and that the structural unit of the crystal has a double chain length. This perpendicular relationship is the distinguishing feature of the "alpha" forms as observed for paraffins, fatty acid esters, etc.

Beta form: The multiplicity of short spacings probably indicates that the hydrocarbon chains are fixed in a definite position as to their relative degree of rotation about the chain axis. The end groups determine a plane and again the structural unit has a double chain length, but from the

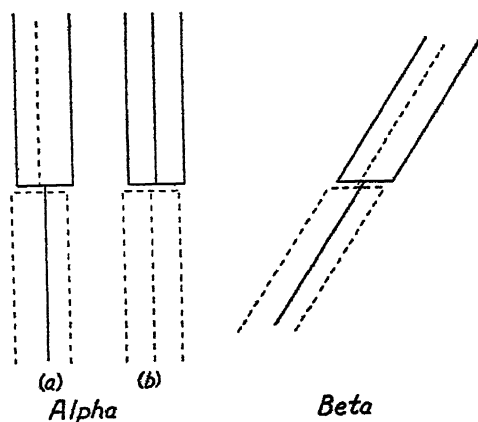


FIG. 5. Malkin's conception of molecular configuration

shorter long spacings that the alpha form exhibits, the hydrocarbon chains must be tilted at an angle of about 65° to the end-group planes (see figure 5). This tilt of the chains is the distinguishing feature of the beta forms and, according to Malkin, it gives the basis for alternation in the homologous series. (The nature of the fourth solid form, β' , mentioned for triglycerides having an odd number of carbon atoms in the chain is presumably like that of the beta form in general features.)

It is possible that mixtures of crystallites of the forms and variations in the degree of structural order in the gamma and alpha forms can give rise to small variations in melting point. Whether the formation of solid solutions, involving different forms, is actually possible has not been settled.

An alternative structure (b), with the three chains of a given molecule side by side, Malkin is inclined to rule out for mechanical reasons that appear in a three-dimensional model.

5. Miscellaneous properties of the triglyceride phases

The literature on volume changes with transformation and melting concerns chiefly the natural and commercial fats. Indeed, the literature on the densities of tristearin in various states has probably not been greatly improved upon since the work of Duffy (11) and Kopp (23) about 1850. Duffy's results are given in table 4.

TABLE 4
Specific volumes of tristearin

THIRD (HIGHEST) MELTING POINT OF SUBSTANCE	TEMPERATURE OF DETER- MINATION	MODIFICATION			
		1 (gamma)	2 (alpha)	3 (beta)	Liquid*
°C.	°C.				
65	15	1.0129			
66.5	15	1.0124			
	15	1.0134	0.9900	0.9825	
69.7	15			0.9824	
	51.5	1.0416		0.9910	
	65.5			1.0069	1.0816
	68.2			1.0260	

* Joglekar and Watson (22) furnish data on the density of several liquid glycerides at several temperatures.

Duffy's conclusions, true or not, were that degree of impurity, as indicated by the first column, little affected the densities of the various forms. From the following brief table a better indication of volume changes can be obtained:

PHASE CHANGE	APPROXIMATE VOLUME CHANGE AT GIVEN TEMPERATURE	TOTAL CHANGE FROM LIQUID
	<i>per cent</i>	<i>per cent</i>
Liquid to gamma.....	4.4	4.4
Gamma to alpha.....	2.3	6.7
Alpha to beta.....	0.8	7.5

The greater density shown for the more stable forms is apparently contrary to the usual experience of tremendous swelling during crystallization and transformation of completely hydrogenated fats approaching tristearin in composition and of tristearin itself (22). Air pockets and vacuoles accompanying the crystallization may be the causes of the discrepancy.

Rao and Jatkar (39) obtained the following values for the heat of crys-

tallization of the gamma and beta forms of tristearin and the beta form of tripalmitin:

GLYCERIDE	HEAT OF CRYSTALLIZATION		HEAT OF TRANSITION
	Gamma form	Beta form	
	cal. per gram	cal. per gram	cal. per gram
Tristearin.....	44.2	61.6	17.4
Tripalmitin.....		62.3	

The following heats of transition of the gamma form to the beta form were obtained from heat of solution data:

Tristearin.....	15.6 calories per gram
Trilaurin.....	15.4 calories per gram

Presumably the heat of crystallization for the alpha form would be intermediate between the values for the gamma and beta forms. It is interesting to note that the heat changes on melting of the two forms, gamma and beta, are roughly proportional to the corresponding volume changes.

Bhide and Bhide (4) found that the dielectric constant of tristearin varies with the previous history of the sample. They found that D of γ -tristearin is lower but near that of the liquid phase. The beta form gives the lowest value of all.

	D
Liquid at 56.8°C.....	2.82
at 90.5°C.....	2.76
Beta form at 31.0°C.....	2.42
at 54.0°C.....	2.45
at 67.0°C.....	2.51

Labrouste (25) and Dervichian (10) have introduced the following evidence to show that the highest melting form, in the case of monomolecular films of triglycerides without external pressure on the film, is the gamma form. It is mesomorphous, that is, it approaches in nature the liquid crystalline state. (1) When sufficient compression piles up visible particles of this form, the particles are observed to melt at the gamma melting point. (2) The gamma form spreads spontaneously on a water surface (unlike the stable form). (3) The "melting" point in the two-dimensional case has been found for several triglycerides to correspond very closely with the three-dimensional gamma melting point (see table 5).

At lower temperatures reversible transformation to another form is observed. The surface area of this form (63 \AA^2 per molecule) corresponds to the x-ray dimensions of the tilted beta form in three dimensions.

With external pressures on the films, evidences of these forms and of one more "solid" form are obtained. This other form, that of minimum film area (55 \AA^2 per molecule), corresponds naturally to the three-dimensional alpha form with its chain perpendicular to the end-group planes.

The fact that the area of the gamma form (71 \AA^2 per molecule) indicates a more tilted structure even than that of the beta form is at first startling, for this means, if Dervichian's argument holds, that in the three-dimensional case during the various transformations a tilted gamma form transforms to an erect alpha form and it in turn to a tilted beta form.

In considering the interrelation of the monomolecular film state to those in three dimensions, account must be taken of a change in chain alinement, for in the case of the monomolecular film, the chains are known to

TABLE 5

GLYCERIDE	MELTING IN FILMS		ORDINARY MELTING	
	Beginning of mesomorphous T_g	Real liquefaction	Vitreous	Stable
	°C.	°C.	°C.	°C.
Tristearin.....	46-7	55	54.5-55	70.8-71.5
Tripalmitin.....	35	45	45 -46.2	64.8-65.6
Trimyristin.....	21	29	32 - 3	55 - 7
Trilaurin.....	0	2-14	14 -16	44.3-46.4

be aligned according to the alternative scheme of figure 5 (Adam (1)), ruled out by Malkin for the three-dimensional case.

V. THE PHASES OF MIXED TRIGLYCERIDES

The experimental investigation (6, 29, 30, 31) of the phase constitution of mixed triglycerides has been limited to glycerides containing only two kinds of fatty acid chains in the molecule, each chain having carbon atoms in even numbers ranging from ten to eighteen.

The mixed triglycerides as a class may exist in at least four different solid modifications. They thus exhibit an even more complex melting and crystallization behavior than the simple triglycerides with an even number of carbon atoms in the chain. The character of the evidence with respect to the existence of and relations between the separate forms is similar to that already presented for the simple triglycerides, and is derived mainly from cooling curves and the x-ray record.

In addition to the gamma, alpha, and beta phases, the mixed triglycerides, as stated above, are able to exist in a fourth solid form. This is called the β' -form, and like all the other forms but beta, is thermodynamically unstable, with melting point intermediate between those of the alpha

and the stable beta forms. With respect to the end-group planes the tilt of the chains is usually, although not always, intermediate between the tilts observed for the alpha and the beta forms. Ordinarily also the x-ray spectrum for the side spacings appears in a narrower band for the β' - than for the β -form.

A. THE SYMMETRICAL MIXED TRIGLYCERIDES

The transformations from one modification to another are more rapid than in the simple triglycerides. Accordingly it is more desirable, and in fact sometimes necessary, to determine the melting points from the heating and cooling curves, rather than in capillary tubes.

These glycerides, like the simple triglycerides, exhibit spherulite formation, but appear to be distinguished from them by a striking rippled effect as the crystallization field is viewed under the microscope.

The symmetrical triglycerides of the following classifications have been studied by Malkin and his coworkers.

1. *Glycerides with fatty acids differing in chain length by two carbon atoms*

(a) Glycerides of the type $\text{CH}(\text{OCOR}')(\text{CH}_2\text{OCOR})_2$, where $R > R'$ by two carbon atoms: e.g., β -palmitodistearin.

(b) Glycerides of the type $\text{CH}(\text{OCOR})(\text{CH}_2\text{OCOR}')_2$, where $R > R'$ by two carbon atoms: e.g., β -stearodipalmitin.

The following distinctions are observed: (1) glycerides of group a tend to solidify more readily in the gamma form; those of group b in the alpha form. (2) Glycerides of group b tend to give more evidence of the alpha form on melting; those of group a apparently tend to transform from the gamma form directly to the β' - or β -form. (3) Whereas the side spacings in the x-ray spectrum for the beta form of glycerides of group a are similar to those for the simple triglycerides, those for glycerides of b are in a narrower band.

There is a remarkable agreement in melting points between corresponding members of groups a and b, e.g., for β -palmitodistearin and β -stearodipalmitin.

2. *Glycerides with fatty acids differing in chain length by four, six, or eight carbon atoms*

(c) Glycerides of the type $\text{CH}(\text{OCOR}')(\text{CH}_2\text{OCOR})_2$, where $R > R'$ by four carbon atoms: e.g., β -myristodistearin.

(d) Glycerides of type $\text{CH}(\text{OCOR})(\text{CH}_2\text{OCOR}')_2$, where $R > R'$ by four carbon atoms: e.g., β -stearodimyristin.

(e) Glycerides of the type $\text{CH}(\text{OCOR}')(\text{CH}_2\text{OCOR})_2$, where $R > R'$ by six carbon atoms: e. g., β -laurodistearin.

(f) Glycerides of the type $\text{CH}(\text{OCOR})(\text{CH}_2\text{OCOF}')_2$, where $R > R'$ by six carbon atoms: e. g., β -stearodilaurin.

(g) The glycerides β -decodistearin and β -stearodidecain.

From Malkin's results it appears that, with the exception of β -palmitodilaurin and β -stearodimyristin, the long spacings of these glycerides in the stable beta form correspond to twice the length of a single molecule

TABLE 6

Melting points of symmetrical and unsymmetrical mixed triglycerides

GROUP	GLYCERIDE	ISO-MER	MELTING POINTS OF SYMMETRICAL MIXED TRIGLYCERIDES				ISO-MER	MELTING POINTS OF UNSYMMETRICAL MIXED TRIGLYCERIDES			
			β	β'	α	γ		β	β'	α	γ
			$^{\circ}\text{C.}$	$^{\circ}\text{C.}$	$^{\circ}\text{C.}$	$^{\circ}\text{C.}$		$^{\circ}\text{C.}$	$^{\circ}\text{C.}$	$^{\circ}\text{C.}$	$^{\circ}\text{C.}$
a...	Palmitodistearin....	β	68	64	56	50	α	65	61	57	50
	Myristodipalmitin..	β	60	55	46	37	α	57	52	47.5	36
	Laurodimyristin....	β	50	45	35	24	α	46.5	42	37	22
	Decodilaurin.....	β	38.5	33	23	8	α	35.5	31	26	5
b...	Stearodipalmitin....	β	68	65	59	49	α	62.5	59.5	55	46.5
	Palmitodimyristin..	β	58.5	55	49	38	α	54	50.5	45.5	34
	Myristodilaurin....	β	48	44	37	24	α	43.5	39	33.5	19
	Laurodidecain.....	β	37.5	34	25	6	α	30	26	17.5	0
c...	Myristodistearin....	β	62.5	59	56	47	α	62	57.5	54	44
	Laurodipalmitin....	β	53.5	50	47	34	α	54	49.5	45	32
	Decodimyristin....	β	43.5	40	37	16	α	43.5	38	32	15
d...	Stearodimyristin....	β	55.5	53	47	33	α	56	52	46	36
	Palmitodilaurin....	β	45.5	42.5	35	19	α	46.5	43	33	20
	Myristodidecain....	β	34	30	21	3	α	34.5	31	20	3
e...	Laurodistearin.....	β	60.5	58	52	36	α		52	47	36
	Decodipalmitin....	β	51.5	48	42	20	α	45.5	41	37	23
f...	Stearodilaurin.....	β	47	43	38	21	α	45	41.5	31	20
	Palmitodidecain....	β	40	36	27	6	α	35	32	24	2
g...	Decodistearin.....	β	57	53	47	30	α	49	46	42.5	33
	Stearodidecain.....	β	44.5	40	34	5	α	41	38	32	13

or four chain lengths (instead of the normal two chain lengths for groups a and b and for the simple triglycerides). In some cases this is also true of the β' -forms. The significance of this behavior is obviously related in some way to the disparity in length between the fatty acid chains.

X-ray spacings and melting points arranged under the foregoing grouping are brought together in table 6.

B. THE UNSYMMETRICAL MIXED TRIGLYCERIDES

These glycerides have been classified after the manner of the classification for the symmetrical glycerides. Important characterizing features of the unsymmetrical triglycerides are their poor crystalline appearance and their slowness of transformation, especially from the β' to the stable β -phase. It is even difficult sometimes to obtain the beta form by crystallizing from solvent (6). Impurity as an explanation for this crystallization behavior can apparently be ruled out, but there is the possibility that these glycerides when synthetic are a racemic mixture of optical isomers.

It will be noted from table 6 that, unlike the case for symmetrical glycerides, no marked difference in side spacings exists between groups a and b. The side spacings of the stable beta forms are very similar to those of the simple triglycerides.

Carter and Malkin confirmed the observation of King and coworkers (2, 3, 32, 41) that the melting points of the unsymmetrical isomer were, in general, a few degrees below those of the symmetrical isomer.

The x-ray and melting point data for the unsymmetrical compounds appear in table 6. As in the case of the symmetrical glycerides where the chain lengths of the acids differ by more than two carbon atoms, the long spacings frequently correspond to four instead of two chain lengths, especially for the most stable beta forms.

New means of identification of glycerides would be of real aid in the study of fat composition. Melting points have been widely used but have usually been subject to some uncertainty, owing to the possible polymorphism. An interesting use of the multiple melting points obtained by Malkin *et al.* is reported in a paper by Hilditch and Maddison (19). A crystallization fraction of cottonseed oil was completely hydrogenated. Other evidence showed it to be a monopalmitodistearin. Careful recrystallizations yielded a substantial portion of a product with the following melting points:

GLYCERIDE	MELTING POINTS, °C.			
	γ	α	β'	β
Palmitodistearin from cottonseed oil.....	50	56	64	67.7
α -Palmitodistearin.....	50	57	61	65
β -Palmitodistearin.....	50	56	64	68

It could "thus be established that in the monopalmitoglycerides of cottonseed oil, the palmitic group is exclusively attached to the beta or central hydroxyl group of the glyceryl radical."

To use the x-ray spacings as a basis for identification would involve

very careful discrimination at the present stage. Consider the four compounds given below:

GLYCERIDE	α LONG SPACINGS	β CHARACTERISTIC SHORT SPACINGS	β LONG SPACINGS	β' LONG SPACINGS
<i>Symmetrical:</i>				
β -Palmitodistearin.....	50.5	4.6, 5.3	44.2	47.5
β -Stearodipalmitin.....	50.2		43.2	44.7
<i>Unsymmetrical:</i>				
α -Palmitodistearin.....	48.8	4.6, 5.3	46.5	44.7
α -Stearodipalmitin.....	47.8	4.6, 5.3	42.5	43.9

β -Stearodipalmitin could be distinguished from the others by the *absence* of the two very characteristic short spacings of the most stable or beta form. α -Palmitodistearin could be distinguished from the others by the greater length of its long spacing for the beta form. The presence of the beta form would have to be definitely assured.

A distinction between β -palmitodistearin and α -stearodipalmitin could be made on the basis of the shorter long spacing of the alpha form of the latter glyceride.

To identify a *single* glyceride by x-ray alone without known materials for comparison would be difficult even if the number of possible glycerides could be limited to a few. However, the first steps have been taken to make such identification a real possibility, and, combined with multiple melting points, the x-ray method should serve in many instances to give far more certain results than have hitherto been possible.

VI. MULTICOMPONENT SYSTEMS OF TRIGLYCERIDES

When it is considered that each individual triglyceride may exist in three or four crystalline forms depending on the treatment of the sample, it is not surprising that so little is precisely established with regard to the phases present in known mixtures of triglycerides or, especially, in natural fats and oils. The fats of commerce are multiple-component systems indeed. The simplest of these chemically are completely hydrogenated products from such materials as soybean oil. These approach tristearin in composition, but by no means closely.

Added to the major complication of polymorphism are the other factors of possible formation of solid solutions (Reimer and Will (40)), uncertainty as to the composition of natural fats or of mixtures of supposedly pure compounds, and a general sluggishness of triglycerides in their approach to equilibrium.

Investigations previous to the work of Kremann (24) appear to have been

of little significance. Indeed, the work of Kremann on the ternary system tristearin-tripalmitin-triolein and on the constituent binary systems, though extensive, cannot now be regarded as of real value. Coming before the elucidation of the polymorphic relationships involved, his results suffer from uncertainty as to the nature of the solid phases obtained. This uncertainty is increased by apparent impurity of materials.

In the opinion of Efremov (12), "all mixtures of tristearin and tripalmitin, independent of composition, repeat entirely the properties of the components", in the sense that all compositions manifested three polymorphic forms. He gave clear evidence that the gamma form, at least, exists through the whole range of possible compositions. His evidence for other forms, by cooling curves and melting points, is less clear. Despite some apparently sound conclusions as to the existence of three polymorphic

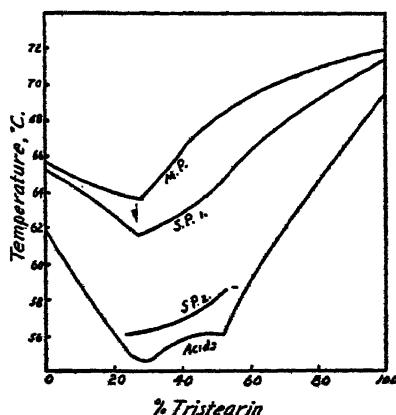


FIG. 6. Mixtures of tripalmitin and tristearin

forms, it is certain from the numerical values of the melting points given that Efremov's materials were not of the purest, at least in the case of tripalmitin.

Probably the best data for the binary system tristearin-tripalmitin are those of Joglekar and Watson (22). These concern, for the most part, the beta form.

In figure 6 their melting-point and main-solidification-point curves indicate pretty clearly that the system exhibits a eutectic in the alpha (Mal'kin's beta) form. Some degree of solid solution formation might be presumed by analogy with other long-chain compounds. Whether a single solid solution phase exists over the whole composition range cannot be decided.

Joglekar and Watson (22) found for a limited range of composition that two solidification points could be obtained, according to whether the cooling

was slow or rapid. They concluded that the lower solidification points referred to the unstable beta (Malkin's gamma) modification. However, it would seem from the magnitude of the numerical values that these second solidification points could more readily refer to the alpha form of Malkin, which otherwise missed the attention of Joglekar and Watson.

That the cooling curve behavior of some of the hydrogenated glycerides of commerce show the same features of polymorphism as tristearin was shown by Nicolet (37).

In a calorimetric study of fat consistency, Straub and Malotaux (46) encountered the phenomenon of polymorphism in connection with a "palmitodistearin" and with coconut oil.

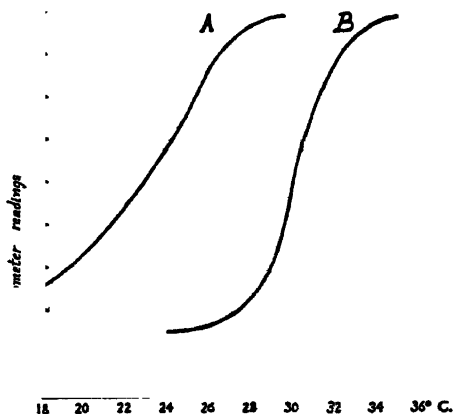


FIG. 7. Effect of the previous treatment of a fat on the course of melting. Curve A, dilatometer curve for cacao butter after chilling and warming immediately; curve B, dilatometer curve for cacao butter after chilling and holding at 24°C. for 15 hr. before warming.

Some very interesting manifestations of polymorphism are to be found in the dilatometric studies of Van Roon (48) and Hofgaard (20).

In figure 7 Van Roon showed how profoundly the previous treatment of a commercial fat, through its effect on polymorphic behavior, can affect the course of melting. The dilatometer curves for cacao butter (A) after chilling and warming immediately and (B) after chilling and holding at 24°C. for 15 hr. before warming (rate of warming, 4°C. every hour) are shown below. Curve A is a temperature-volume curve of cacao butter consisting largely of unstable low-melting modifications. Curve B shows the behavior of the same fat after transformation of much of the unstable to more stable forms.

Similar curves for many fats have been obtained by Hofgaard (20). He

has pointed out that the state and physical behavior of a fat may depend in large measure on the ability of the triglyceride to crystallize in various modifications. By means of dilatometric technique the divergence of fats from the state of equilibrium at some given temperature can be observed, and thus the conversion of one phase to another may be followed. (If the fats are not 100 per cent solid, the density changes due to polymorphism may be confused with density changes resulting simply from readjustment of solid-liquid proportions.)

Hofgaard noted that the phenomenon of polymorphism is clearly traceable in beef tallow, cacao butter, shea butter, palm oil, and Illipe fat, but coconut and palm kernel oils show little or no signs of the appearance of unstable forms (perhaps an indication of the greater rate of transformation in fats of low molecular weight). With increased degree of hardening (hydrogenation), the tendency to solidify in unstable forms is more pronounced. (This may indicate merely that, as the melting point rises, so also does the temperature where the transformation rate becomes rapid increase.)

The clarification of the crystallization behavior of fats on a theoretical and practical basis is not yet far advanced, but it is definitely beyond the confused stage of less than a decade ago.

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SURFACE FILMS OF FATTY ACIDS, ALCOHOLS, AND ESTERS¹

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I. INTRODUCTION

Important information concerning the molecular structure of fats and oils has been obtained by the use of x-rays, of electron diffraction, and of surface films. In the applications of these substances in industry and in everyday life the intermolecular structure, that is, the pattern according to which the molecules are fitted together, is fully as important as the structure of each individual molecule. While the x-ray method gives remarkable results if applied to crystalline material in bulk, and some information of value with liquids, the investigation of two-dimensional systems reveals the superiority in this case of the use of electron diffraction and the study of surface films. Beams of slow electrons are best suited to the study of extremely thin films, but unfortunately no investigator has as

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yet mastered both the technique and the theory of the use of slow electrons, that is, of energies from 10 to 1000 electron volts (e.v.), for this purpose. What has been revealed by x-rays, by electrons of energies of the order of 40,000 e.v., and by the study of surface films shows that within the limits of our power of interpretation all three methods agree with each other whenever they have anything in common.

II. THE SPREADING OF OIL FILMS ON WATER

A. Oxidized oils

If a drop of fresh Nujol, a heavy hydrocarbon oil, is put on a clean surface of water it does not spread, but remains as a thick lens. If the Nujol has been oxidized by air by stirring it in an evaporating dish over a flame, it is found to spread as a uniform polymolecular film over a considerable area. If, for example, the area of the surface of the water in a rectangular trough is $5 \times 10^3 \text{ cm.}^2$, a small drop of this oil spreads over the surface to form an invisible film, which, on moderate decrease of area on compression by the use of a movable barrier, exhibits a uniform light yellow color in white light. On compression the color changes to dark yellow, gold, red purple, blue, and green, and these colors then repeat themselves as the spectrum moves through four or more orders, but in the higher orders the colors red and green become much the most prominent. On further thickening, the film becomes colorless. An experiment such as is described involves films of a uniform optical thickness between a few hundred and several thousand Ångström units. The limits of thickness are much greater than this.

Such polymolecular liquid films may be designated as duplex, since their upper and lower surfaces are far enough apart to make their surface energies entirely independent of each other. Thus the total free energy, γ_a , of unit area of the duplex film of a liquid *b* on a liquid *a* is given by the expression:

$$\gamma_a = \gamma_b + \gamma_{ab} \quad (1)$$

Such a duplex film is, in general, unstable. Thus an oil film on water which exhibits a single diffraction color is always found to segregate into regions of varied thickness, and therefore of varied colors, at the end of a period which varies from a few seconds to a few weeks. In order to understand what occurs, a knowledge of the thermodynamics of films is essential.

If a crystal of an organic acid, an alcohol, or an ester is placed on the surface of clean water, it is found to spread as a monolayer. Oleic acid seems to spread to some extent as a duplex film, but the monolayer spreads

more rapidly and therefore ahead of the duplex film, which changes rapidly to a monolayer and lenses.

B. General theories

There are three theories of the spreading of oil on water which should be considered.

1. Rayleigh's theory (18, 22, 23): All liquids spread on the surface of clean water. To prevent spreading, it is necessary to have a foreign substance on the surface.

2. Langmuir's theory (16, 17): Non-polar liquids, such as the hydrocarbons, do not spread on water, but if the molecules contain a polar group and a non-polar group, the liquid will spread.

3. The general thermodynamic theory of Harkins (8, 11), which is not limited, but applies to all liquids, to the spreading of solids on liquids, and to the spreading of mobile films on solids. The theory is based on the concept that there are two types of spreading:² (A) Duplex (D) spreading: a liquid, such as benzene or the oxidized Nujol already considered, may spread as a duplex film. The thermodynamic relations indicate that this is unstable and always transforms into a non-duplex film (a monolayer) and a lens or lenses. (B) Non-duplex (or M) spreading: a non-duplex film may spread directly from a three-dimensional solid or liquid, or it may be formed by the transformation of a duplex film. All non-duplex films of known thickness are monolayers.

Duplex spreading: The theory of the spreading of a duplex film may be stated in the following abbreviated form: If a drop of an insoluble oil (b) is placed on a clean water (a) surface, it may or may not spread. The condition which determines whether or not the oil spreads is exceedingly simple. *If the oil likes itself (W_a) better than water (W_b) it will not spread, while if it likes the water better than itself, it will spread.* In order to use this relation in a quantitative sense it is essential to express the term "likes" as a thermodynamic quantity, which is done in the following equation:

$$S_{b/a} = -(\partial F / \partial \sigma)_{p,T} = W_A - W_b \quad (2)$$

Here $-(\partial F / \partial \sigma)_{p,T}$ is the rate of decrease of the free energy (F) of the system with increase of area (σ) of the duplex film, and the concomitant decrease in the area of the clean surface of the water. W_A is the work

² Hardy (5) also assumed two types of spreading: primary, which corresponds to non-duplex, and secondary, which corresponds to duplex. One of the principal differences is that Hardy assumed non-duplex films to be moderately thick, while here they are assumed to be monomolecular in general.

required to pull the oil and water apart over an area of 1 sq. cm., and W_c is that required to rupture a bar of oil with a cross section of 1 sq. cm. of surface of the oil (figures 1 and 2). A consideration of these processes gives values for W_A and W_c as follows:

$$W_A = \gamma_a + \gamma_b - \gamma_{ab} \quad (\text{Dupr 's equation (3)}) \quad (3)$$

$$W_{c_b} = 2\gamma_b \quad (4)$$

so

$$S_{b/a} = \gamma_a - (\gamma_b + \gamma_{ab}) \quad (5)$$

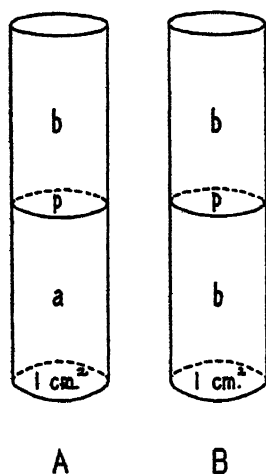


FIG. 1

FIG. 1. (A) If the liquid b is pulled from the liquid a at the plane p , the increase in free energy is $\gamma_a + \gamma_b$ in the ideal case in which the liquids are completely separated from each other. There is a concomitant decrease of free energy equal to γ_{ab} , due to the destruction of the interface of ab . The work done (W_A) equals the net increase in free energy, or $W_A = \gamma_a + \gamma_b - \gamma_{ab}$. (B) If the liquid b is pulled apart at the plane p , the increase in free energy is $2\gamma_b$, or $W_c = 2\gamma_b$.

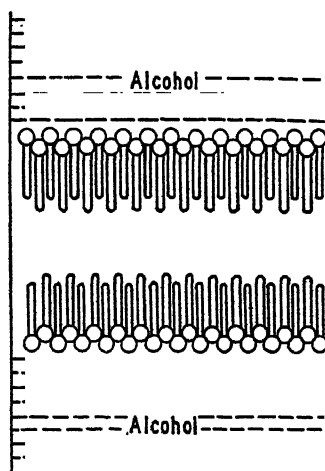


FIG. 2

FIG. 2. Idealized diagram illustrating the separation of a bar of liquid alcohol into two parts. The molecules are represented as already oriented at the interface, since the break is well under way. This diagram indicates that in such an ideal case the energy of separation of a moderately long chain alcohol should be practically equal to that of a hydrocarbon of the same molecular size, except for the relatively small energy of orientation of the molecules at the interface before the actual separation has occurred.

C. Benzene (figure 3)

In order to obtain a better idea of the extent to which each of the three theories is valid, the discussion given below considers the spreading of

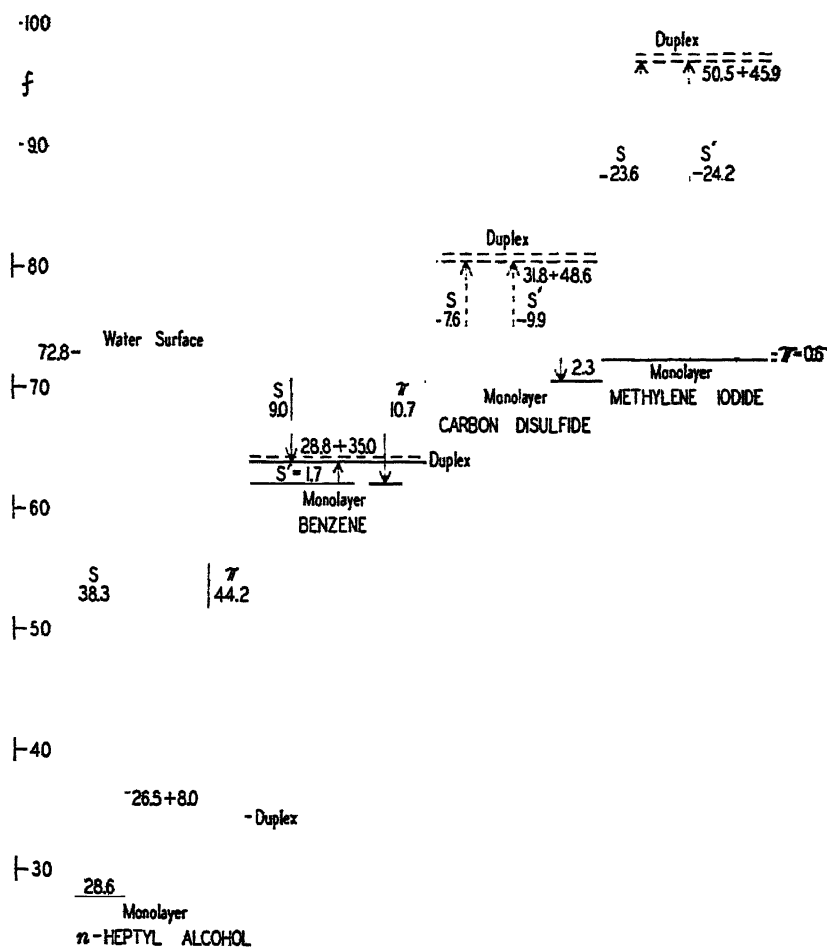


FIG. 3. Free-energy diagram for the spreading of oils on water. Any film the free-energy level of which is above that of water (72.8 ergs cm.⁻² at 20°C.) cannot spread, and any film the level of which is below that of water may spread spontaneously on water. Benzene, a non-polar liquid (isopentane has practically the same levels), should, according to the diagram, be able to spread either as a duplex film or as a monolayer, but only the latter is stable. For carbon disulfide, a non-polar liquid, the duplex film is at a free-energy level 7.6 ergs cm.⁻² higher than that of a clean water surface, so a duplex film is not formed by spreading. The free-energy level of the gaseous monolayer is, however, 2.3 ergs cm.⁻² below that of the water surface, which rapidly attains equilibrium with the lens and the vapor in a saturated atmosphere. The film pressure, as for benzene, is given by the difference in level between the clean water surface and that of the monolayer. Methylene iodide, even though it is highly polar, does not spread as a duplex film, and its monolayer gives only a small film pressure (0.6 dyne cm.⁻¹).

three liquids,—benzene, carbon disulfide, and a paraffin oil of high molecular weight.

For dry benzene on a clean water surface, what may be termed the initial spreading coefficient has the value

$$S_{b/a} = \gamma_a - (\gamma_b + \gamma_{ab}) = 72.8 - (28.9 + 35.0) = 8.9 \text{ ergs cm.}^{-2}$$

That is, if the area of the duplex film increases by 1 cm.² and that of the clean surface of the water decreases by this amount, there is a decrease in free energy of 8.9 ergs, which is a considerable decrease and indicates that benzene should spread readily to form a duplex film.

However, since the extremely thin duplex film of benzene becomes saturated with water very rapidly, the coefficient of spreading, which may be designated as the semi-initial coefficient, becomes

$$S_{b'/a} = \gamma_a - (\gamma_{b'} + \gamma_{a'b'}) = 72.8 - (28.8 + 35.0) = 9.0 \text{ ergs cm.}^{-2} \quad (6)$$

where the prime indicates that *b* is saturated with *a*. The value of this spreading coefficient indicates that wet benzene also spreads on a clean water surface to form a duplex film.

If *b* and *a* are mutually saturated, then

$$S_{b'/a'} = \gamma_{a'} + (\gamma_{b'} - \gamma_{a'b'}) \\ = 62.2 - (28.8 + 35.0) = -1.6 \text{ ergs cm.}^{-2} \quad (7)$$

This may be designated as the *final spreading* coefficient. Its negative value indicates that benzene will not spread over the surface of water if the liquids are mutually saturated. An inspection of the numerical terms involved shows that the whole change between $S_{b'/a}$ and $S_{b'/a'}$ is due to a decrease of 10.6 ergs cm.⁻² in the free surface energy of the surface of water. Now if the free surface energy of the water saturated with benzene is subtracted from that of the clean water, there is obtained as the difference (π_s)

$$\gamma_w - \gamma_s = \pi_s \quad (8)$$

where, if surface tensions instead of free surface energies are considered, π is by definition the film pressure, and the subscript in π_s and γ_s indicates that the film of the oil *b* is in equilibrium with a lens of the oil.

Now equation 6 minus equation 7 gives

$$S_{b'/a} - S_{b'/a'} = \gamma_a - \gamma_{a'} = \gamma_w - \gamma_s = \pi_s \quad (9)$$

or the difference between the semi-initial and the final coefficients of spreading is equal to the film pressure. It is shown above that for benzene on water this difference is 10.6 ergs cm.⁻², which is therefore the value the film pressure of the non-duplex film of benzene on the water.

To obtain this thin film a clean surface of water in a large crystallizing dish may be partly covered with a layer, that is, a large lens, of benzene. If the curvature of the surface of the water is adjusted to give such a result, the lens or layer (*ca.* 2 mm. thick) will form an annular ring in contact with the outer edge of the dish, while in the center a circular hole will be formed. According to Langmuir there is no benzene film in this hole, but the vertical type of film balance, or the ring method, may be used to test this, and such a measurement gives a film pressure of 10.6 dynes cm.^{-1} . Thus the surface of the water in the hole is covered with a monolayer which gives this high pressure, corresponding to about 200 atmospheres. Obviously this monolayer is in equilibrium with the large lens.

D. Carbon disulfide (figure 3)

For carbon disulfide on water the semi-initial coefficient of spreading is

$$S_{b'/a} = \gamma_w - (\gamma'_o - \gamma'_{wo}) = 72.8 - (31.8 + 48.6) = -7.6 \text{ ergs cm.}^{-2}$$

while the final coefficient is

$$S_{b/a'} = 70.5 - (31.8 - 48.6) = -9.9 \text{ ergs cm.}^{-2}$$

or

$$\pi_e = S_{b'/a} - S_{b/a'} = 2.3 \text{ ergs cm.}^{-1}$$

Thus the values of the initial and semi-initial spreading coefficients predict that carbon disulfide will not spread as a duplex film, but the difference between the semi-initial and final spreading coefficients indicates that it does spread as a monolayer, which has an equilibrium film pressure of 2.3 dynes cm.^{-1} . Presumably this monolayer is a gaseous film. Obviously it may have any film pressure less than 2.3 dynes cm.^{-1} if it is not in equilibrium with a lens of the liquid and the vapor from the liquid.

Methylene iodide, a non-spreading oil in the sense that its spreading coefficient has a very large negative value ($S_{o'/w} = -23.6$ and $S_{o'/w'} = -24.2$), nevertheless gives a non-duplex film or monolayer with a film pressure of 0.55 dyne cm.^{-1} .

E. Paraffin oil of very high molecular weight

A paraffin oil may have a molecular weight so high that at 20°C. it gives almost no vapor pressure in three dimensions. Such an oil gives a high negative spreading coefficient, so it does not spread as a duplex film. Its cohesion is so large that it does not even spread as a gaseous monolayer, at least not sufficiently to give a measureable film pressure with the ordinary type of film balance. Such an oil may be considered as non-spreading.

III. TRANSFORMATION OF A DUPLEX FILM INTO A MONOLAYER AND A LENS

The examples given in section II show that in each case the free surface energy of the monolayer is less than that of the duplex film, provided the duplex film can spread at all. This is a general relation, so the duplex film always changes into a monolayer and a lens (or lenses if the time is not sufficiently long to give a single lens). If a duplex film cannot form, then if any film is produced it is a monolayer. Such a monolayer may be formed even if the initial spreading coefficient is negative, but may not

TABLE 1

A. Initial spreading coefficients of a few oils on clean water at 20°C.

MOLECULES WITH POLAR GROUPS	<i>S</i>	PARAFFINS	<i>S</i>	NON-POLAR AROMATIC HYDROCARBONS	<i>S</i>
<i>n</i> -Octyl alcohol.....	35.7	Isopentane.....	9.4	<i>p</i> -Cymene.....	10.1
Heptaldehyde.....	32.2	Hexane.....	3.4	Benzene.....	9.8
Oleic acid.....	24.6	Diisooamyl.....	3.7	<i>o</i> -Xylene.....	6.9
Ethyl nonylate.....	20.9			Toluene.....	6.8
<i>o</i> -Nitrotoluene.....	4.1			<i>p</i> -Xylene.....	6.7
Nitrobenzene.....	3.8			Mesitylene.....	5.6

LIQUIDS WITH NEGATIVE COEFFICIENTS	<i>S</i>	LIQUIDS WITH NEGATIVE COEFFICIENTS	<i>S</i>
Ethylene dibromide.....	-3.2	Monoiodobenzene.....	-8.7
<i>o</i> -Monobromotoluene.....	-3.3	Bromoform.....	-9.6
Carbon disulfide.....	-4.2	Acetylene tetrabromide.....	-15.6
Long-chain paraffins.....	-6.9	Methylene iodide.....	-26.5

B. Some final spreading coefficients of oils on water

OIL	<i>S</i>	OIL	<i>S</i>
Benzene.....	1.5	<i>n</i> -Heptyl alcohol.....	-5.9
Carbon disulfide.....	-10.0	Isoamyl alcohol.....	-2.6
Methylene iodide.....	-24.2		

give a measureable film pressure if the oil is non-polar and does not give a measureable three-dimensional vapor pressure.

If the atmosphere above an oil film is supersaturated with oil, the material condensed on the monolayer does not thicken it, but any excess deposited on the monolayer either evaporates or goes *into* the monolayer. As a result of this latter process, either the monolayer expands, or an equal amount of material in the monolayer is forced into the lens.

The rate of spreading of an oil on water increases with the magnitude of the spreading coefficient and with a *decrease* in the viscosity of the layer which spreads. The values of the initial spreading coefficients of a

few oils are given in table 1. The final spreading coefficient of an oil on water is always negative, so no oil will spread over its equilibrium monolayer. The initial and final coefficients for water on oil are always negative, so water will not spread over the surface of an oil.

Since (1) many hydrocarbon oils, which do not contain polar groups, nevertheless spread on water to duplex films and then to monolayers, and (2) substances whose molecules contain a strongly polar group do not spread if the hydrocarbon fraction is too large, it is apparent that the presence or absence of a polar group is not the true criterion for spreading or non-spreading. Examples of the second type are arachidic acid (twenty carbon atoms) and acids having longer normal paraffin chains. These substances are solids on account of the higher cohesion due to the longer chains.

IV. PHASE RELATIONS OF MONOLAYERS

Monolayers are found to exhibit the three types of phases found in three-dimensional systems: (1) gas, (2) liquid, and (3) vapor. The characteristics of the phases of the two-dimensional systems may be exhibited by pressure-area (π - σ) diagrams (figures 4, 5A, and 5B), which exhibit relations similar to those represented in three dimensions by p - v diagrams (10, 14).

Within a suitable temperature range many different polar-non-polar oils are found to give a π - σ diagram of the type shown in figure 4. The temperatures in the figure are correct for pentadecylic acid (fifteen carbon atoms), but are lower for acids with a lower, and higher for those with a higher number of carbon atoms per molecule. With alcohols the temperatures are much higher. At very large molecular areas, of the order of $50,000 \text{ \AA}^2$, the film of pentadecylic acid obeys the law $\pi\sigma = kT$, where σ is the area and k is Boltzmann's constant. This is the law of a perfect gas. As the film is compressed to lower areas this two-dimensional gas becomes more and more imperfect, but the film remains homogeneous. This can be shown by the use of a small silver electrode, coated with polonium, placed a short distance above the surface of the water. This is connected through any type of electrometer to a calomel cell, the arm of which dips under the solution in the trough of the film balance.

The difference between the potential of the electrode when it is over a clean water surface, and when a film is on the surface underneath the electrode, is designated as the surface potential. The sign of this potential is taken as positive when films of organic acids, alcohols, etc. are present, but the salts of the acids exhibit a slight negative potential. With certain films the surface potential is as large as 950 millivolts.

The surface potential, if only a single organic substance is present, is

roughly proportional to the number of molecules per unit area, and since this number increases with the pressure, the surface potential also increases. It was found by Harkins, Ries, and Carman (13) that condensed films of tetrahydro-*d*-pimeric acid, a saturated compound, exhibit a normal increase of from 203 to 220 millivolts as the molecular area is decreased from 54 to 46 Å.² The structure of *d*-pimaric acid is the same, except for two double bonds far from the carboxyl group.

With the film of this compound the same decrease of area *decreases* the film potential from 108 to 94 millivolts. The double bonds reduce the

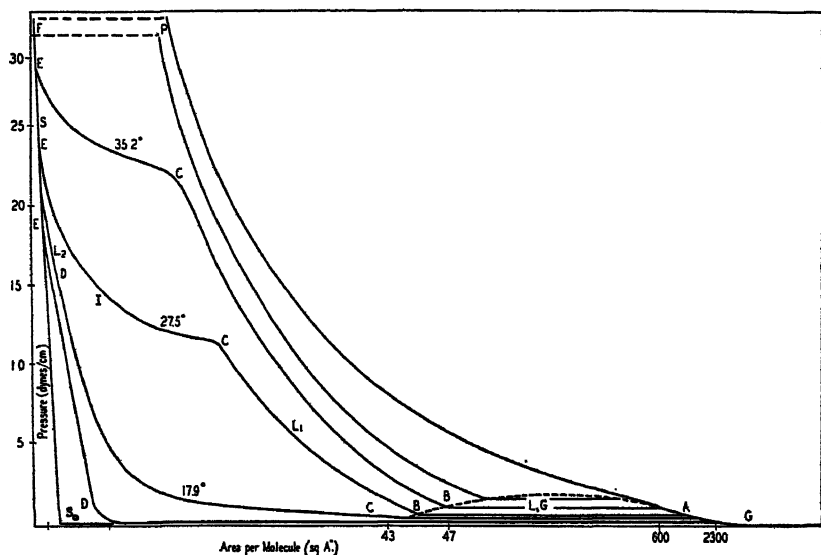


FIG. 4. General phase diagram of a monolayer at a temperature below the critical temperature for the formation of a liquid expanded (L_1) film. Phases: FE or S , solid; ED or L_2 , liquid condensed; DC or I , intermediate; CB or L_1 , liquid expanded; BA or L_1G , gaseous film and L_1 film in equilibrium; G , gaseous film.

potential due to the carboxyl group dipole by more than 50 per cent, which shows that their mutual dipole is opposed to that of the carboxyl group. The decrease of the potential on compression shows that a closer packing of the molecules, which presumably causes them to stand more upright on the surface, brings these dipoles more into opposition.

If at any given film pressure there are islands of liquid film in a sea of gaseous film, a survey of the surface, by moving the electrode over it, shows that the surface potential is much higher over an island than when there is only a gaseous film underneath. Such a survey may fail to reveal non-homogeneities, provided small islands are uniformly distributed over the whole area.

At 17°C. the gaseous film of myristic acid becomes entirely condensed to a liquid (L_1) state at a molecular area of 45 \AA^2 . At somewhat higher areas the film is heterogeneous (region L_1G of figure 4), and this is shown by an electrical survey of such a film made at a mean molecular area of 56.5 \AA^2 . The numbers represent the distribution of the film potential over an area about $25 \times 50 \text{ cm}$. The values vary from zero, where only the gaseous film was present, to 199 millivolts in the region where the "island" was the most continuous. At an area of 37.1 \AA^2 the film is in the inter-

TABLE 2
Surface potentials of myristic acid films at 23°C. (12)

AREA	SURFACE POTENTIALS IN MILLIVOLTS						
Gaseous and liquid expanded (L_1) film							
56.5 Å. ²	10	22	31	43	90	165	158
	0	15	30	38	164	160	156
	5	35	51	165	167	165	165
	48	98	166	174	171	176	164
	145	148	170	169	170	103	199
Liquid expanded film							
37.1 Å. ²			175	170	170	160	157
			172	165	168	160	155
			169	166	167	164	158
			166	169	188	165	155
			163	167	190	195	
Intermediate film							
25.0 Å. ²				255	248	255	281
				255	248	255	245
				255	252	258	243
				254	277	261	238
				253	254	261	256

mediate state, and at 25.0 \AA^2 it is still in the same state. Stearic acid in the L_2 or liquid condensed state gives a potential of about 410 millivolts at 17°C.

At 14.5°C. a monolayer of pentadecylic acid begins to condense to the liquid (L_1) state if compressed below 2300 \AA^2 , or at 24°C. if below 800 \AA^2 (figure 4).

The transition of a monolayer from a gaseous to an L_1 liquid state is an ordinary change of the first order, accompanied by a latent heat of phase transformation. Thus it is analogous to the condensation of a gas to a liquid in a three-dimensional system.

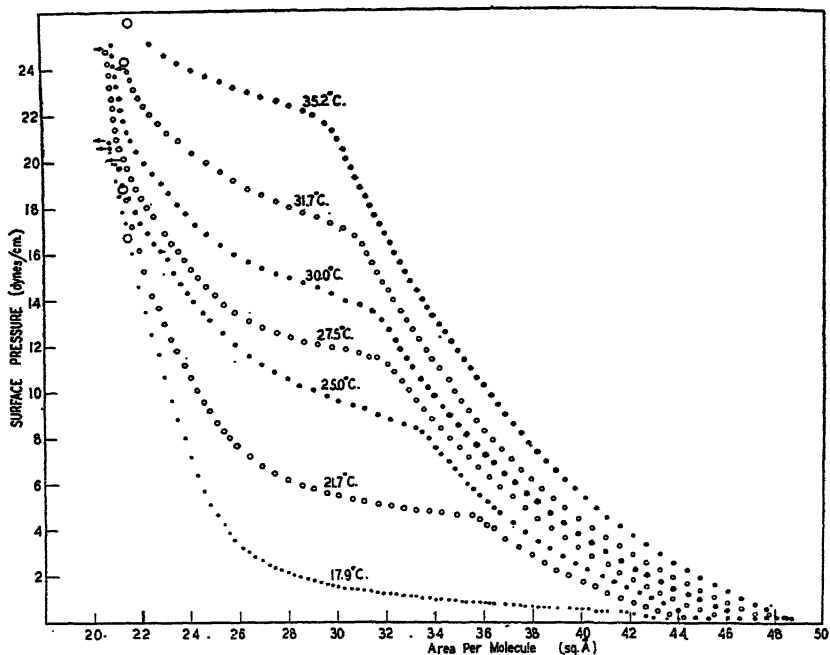


FIG. 5A. Liquid films of pentadecylic acid

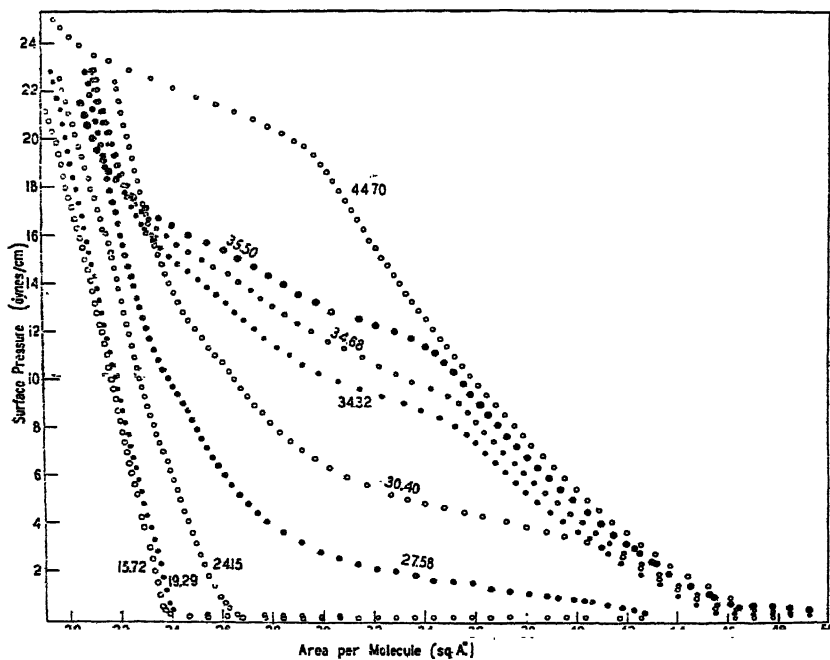


FIG. 5B. Liquid films of palmitic acid. Above a pressure of 13 dynes some of the curves are affected by a slight collapse of the film.

TABLE 3

*Two-dimensional phases and probable order of changes between them*ORDER OF CHANGE BETWEEN
TERM

Classification A

- I. Gas, *G*. Includes compressed vapors such as the "vapor expanded" film of Adam

Perfect gas: $\pi\sigma = kT$

Imperfect gas: minimum of same order as for L_1 films: e.g., 0.04 for ethyl palmitate at 25°C., 34 Å.², and 14 dynes cm.⁻¹

I and II: first

- II. Liquid, L_1 (*BC*). "Liquid expanded" of Adam.

Liquid of high compressibility

$$-\kappa = 2 \text{ to } 7 \times 10^{-2}$$

$$h_s = 50 \text{ ergs cm.}^{-2}$$

II and III: resembles diffuse first-order change of phase, but is probably second order

- III. Intermediate (*I*) or transition (*CE*). Liquid of extremely high compressibility

Intermediate, *I*.

Maximum $-\kappa$ of order of

$$2 \text{ to } 5 \times 10^{-1} \text{ for normal long-chain acids}$$

$$2.2 \times 10^{-1} \text{ for normal long-chain esters}$$

Minimum same as $-\kappa$ of L_2 film: $h_s = 300 \text{ ergs cm.}^{-2}$, which is nearly constant at the higher areas, but decreases rapidly to a low value as transition to next phase is approached

III and IV: third

- IV. Liquid, L_2 (*DE*). "Liquid condensed" of Adam.

Liquid of low compressibility

$$-\kappa = 5 \text{ to } 10 \times 10^{-3}$$

$$\log \eta = \log \eta_0 - k\pi : h_s \text{ small}$$

IV and V: second

- V. Solid, *S* (*EF*)

$$-\kappa = 7 \text{ to } 9 \times 10^{-4}$$

$$h_s = 200 \text{ to } 700 \text{ ergs cm.}^{-2}$$

Classification B: same as A, except as indicated below

- III. Intermediate

(a) Transition, *T*

(b) Liquid, L_2

- IV. Solid

A new, very highly condensed phase, designated as the LS phase (the *L* indicating that it has a much higher fluidity than the liquid from which it is formed, and the *S* that it has the compressibility of the solid phase) seems to be formed from the liquid L_2 phase by a first-order change. This is the only first-order change known in two-dimensional systems, except when a gaseous phase is condensed.

On compression the pressure of the liquid (L_1) film increases along a curve which has almost the form of an equilateral hyperbola, as may be seen more clearly in an enlarged section of figure 4, as in figure 5A.

At C (figure 4) there is a phase transformation of a peculiar type to the *intermediate* (I) liquid state. Whether this is a diffuse first-order change, or one of the second order, is not known. Just above the kink point the π - σ curve is almost, but not quite, horizontal. This is a region of considerable hysteresis.

At D (figure 4) there is a third-order transition to a condensed liquid (L_2) monolayer, and at E , a second-order transition to a solid.

Table 3 lists the five phases which may be formed on the compression of a monolayer, together with the probable order of the phase change between them. The compressibility (κ) is characteristic of the phase, as is the increase of enthalpy (h_s) which accompanies the spreading over unit area. This has almost the same value as the heat absorbed.

V. ESTERS AND METHYL KETONES (10)

The substitution of a methyl or an ethyl group for the acid hydrogen of a long-chain acid reduces the cohesion in the film. This gives a remarkable effect on the general phase diagram, since it may entirely eliminate the liquid expanded (L_1) phase, which is replaced by a gaseous monolayer, and this exhibits a hyperbolic π - σ relation. It is distinguished from the L_1 state only by the absence of the first-order transition from gas to liquid (L_1), and thus the heterogeneous region (L_1G) of figure 4 does not appear. Figures 6 and 7 represent the variation of the film pressure with molecular area of the ethyl esters of the normal acids from myristic to margaric at 25°C. and 15°C., respectively. According to Adam (1) the esters of all of these acids give high-pressure gaseous or *compressed vapor films* at 15°C. with the exception of ethyl margarate, which transforms from a low-pressure vapor directly to a condensed liquid state. If this is true, then at 25°C. every one of the four esters exhibits the compressed vapor state. In all cases increase of pressure causes the vapor phase to undergo a phase change into the intermediate state, provided it does not collapse, that is, if it does not exhibit instability with reference to the third dimension. At 25°C. this collapse occurs with the myristate and the pentadecylate at pressures of 16.5 and 17.5 dynes cm.⁻¹, respectively, and at a molecular area of about 32 Å.² At 15°C. none of the gaseous films collapses. The myristate undergoes a phase change to the intermediate state at about 32 Å.² and a film pressure of 16.2 dynes cm.⁻¹

With the exception of the margarate at 15°C., all of the esters are at

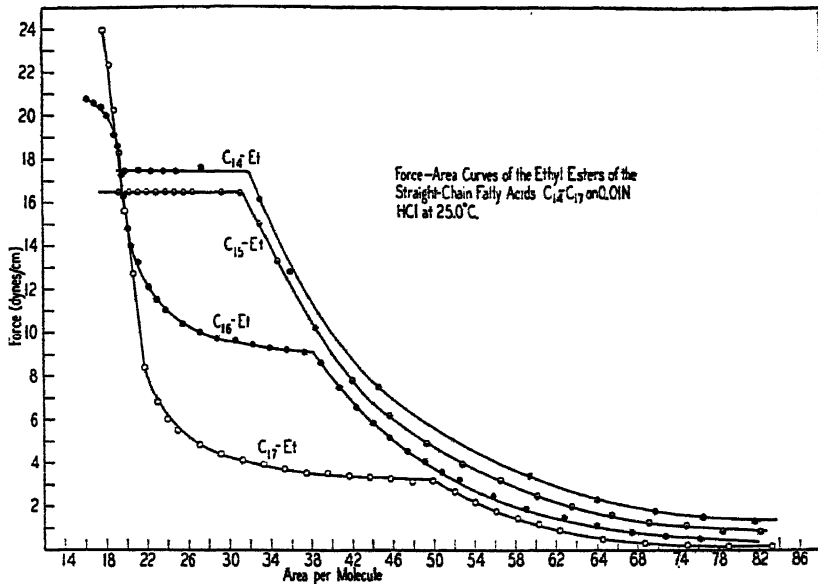


FIG. 6. General phase diagram at temperatures above T_c for the L_1 phase: ethyl esters at 25°C.

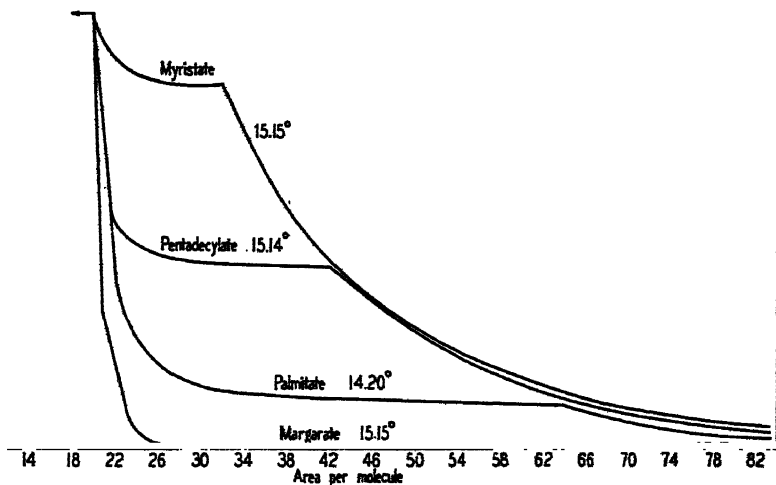


FIG. 7. General phase diagram at temperatures above T_c for the L_1 phase: ethyl esters at about 15°C.

temperatures above the critical temperature for the phase transition from gaseous film to the liquid (L_1) state.

It is obvious, from the form of the curves, that in the relation

$$(\pi - \pi_0)(\sigma - \sigma_0) = kT$$

the value of π_0 is much smaller and that of σ_0 somewhat smaller for the compressed vapors than for expanded (L_1) liquids the molecules of which contain the same number of carbon atoms.

Langmuir (17) considers that these esters do not form a liquid condensed (L_2) phase, but the straight lines of figures 6 and 7, which when extrapolated to zero pressure give a molecular area of 24 \AA^2 , seem to represent liquid phases. The extrapolated area for the solid phase is at zero pressure only about 21 \AA^2 , and the determined value at $19 \text{ dynes cm.}^{-1}$ is 20 \AA^2 .

The compressed vapor films undergo a phase transformation to the intermediate phase at a much higher molecular area than with the acids. For example, at 14.2°C . ethyl palmitate undergoes this transition at a molecular area of 64 \AA^2 ($\pi = 2 \text{ dynes cm.}^{-1}$), while at even a much lower pressure the similar change for a long-chain paraffin acid does not occur much above 42 \AA^2 . Presumably this is due to the greater probability that the molecules of the ester lie flat on the surface, owing to the difficulty of making a sharp bend in the molecule at the polar group. In a condensed state the polar group is kept farther from the water by the ethyl group of the ester than by the proton which it replaces in the acid. When a greater molecular area is available, many of the polar groups of the ester can come in contact with the surface of the water, and thus orientation perpendicular to the surface becomes much less probable. If ethyl palmitate is in the compressed vapor state at 10°C ., it should undergo the transition to the intermediate liquid state at about 80 \AA^2 , or four times the area occupied in the condensed state. Even at this area, however, not all of the molecules can lie flat, since this requires 115 \AA^2 per molecule.

The low area to which the compressed vapor state may persist is quite remarkable. Thus at 25°C . ethyl myristate remains as a vapor until its area is only 32 \AA^2 , or not much above one-fourth that required for the flat orientation.

Another feature of the ester films is the very high compressibility (e.g., $-\kappa = 2.2$ for ethyl palmitate at 15.2°C .) exhibited at molecular areas slightly below that for the transition from vapor to intermediate film. Thus this transition is much more nearly of the first-order type than when long-chain acids are involved.

VI. CONDENSED LIQUID (L_2) FILMS OF ACIDS, ALCOHOLS, AND ESTERS (19)

As the molecular area of an intermediate surface phase is decreased its pressure rises more and more rapidly, until at a point D the π - σ curve begins to be linear. The linear region represents what is designated as

the condensed liquid phase. This has a relatively low compressibility, of the order of 0.008 for acids and 0.005 for alcohols, if the hydrocarbon chains are long.

The viscosity relations (2, 9) of these liquid monolayers are of considerable interest. Figure 8 exhibits the effect of pressure and of chain length upon the viscosity of the normal-paraffin-chain acids. For the eighteen-, nineteen-, and twenty-carbon-atom acids the logarithm of the

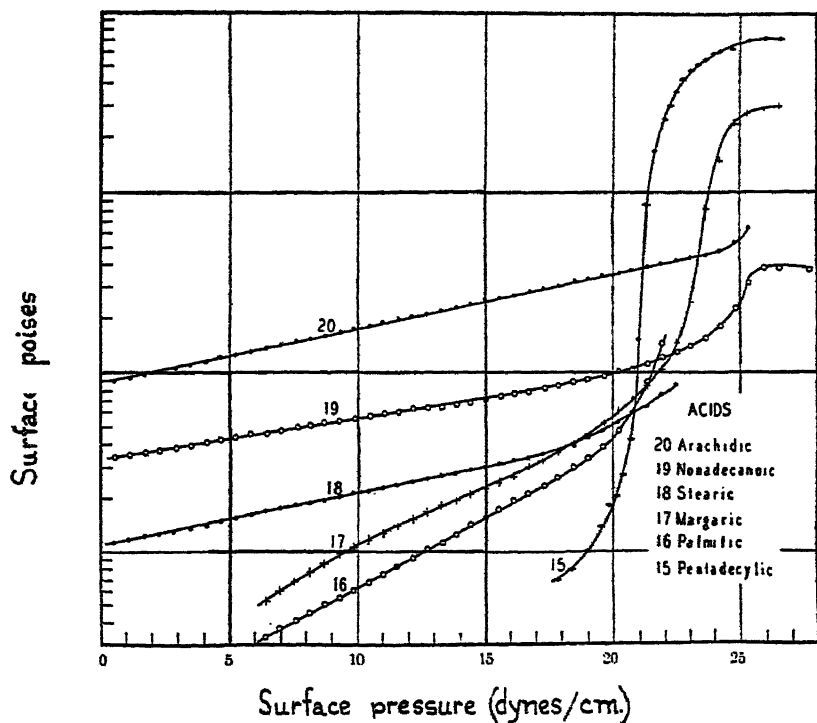


FIG. 8. Viscosity-pressure relations of monolayers of the normal long-paraffin-chain acids

viscosity of the liquid monolayers increases by a constant amount per carbon atom. The effect of pressure is given by the following equation:

$$\log \eta = \log \eta_0 + k\pi$$

where η represents the viscosity of the film. The viscosity increases much more rapidly than this as the pressure approaches that for the transition: Liquid (L_2) \rightarrow Solid. At the freezing point the viscosity is increasing with great rapidity.

The relations for the alcohols (4) are much the same, except that the

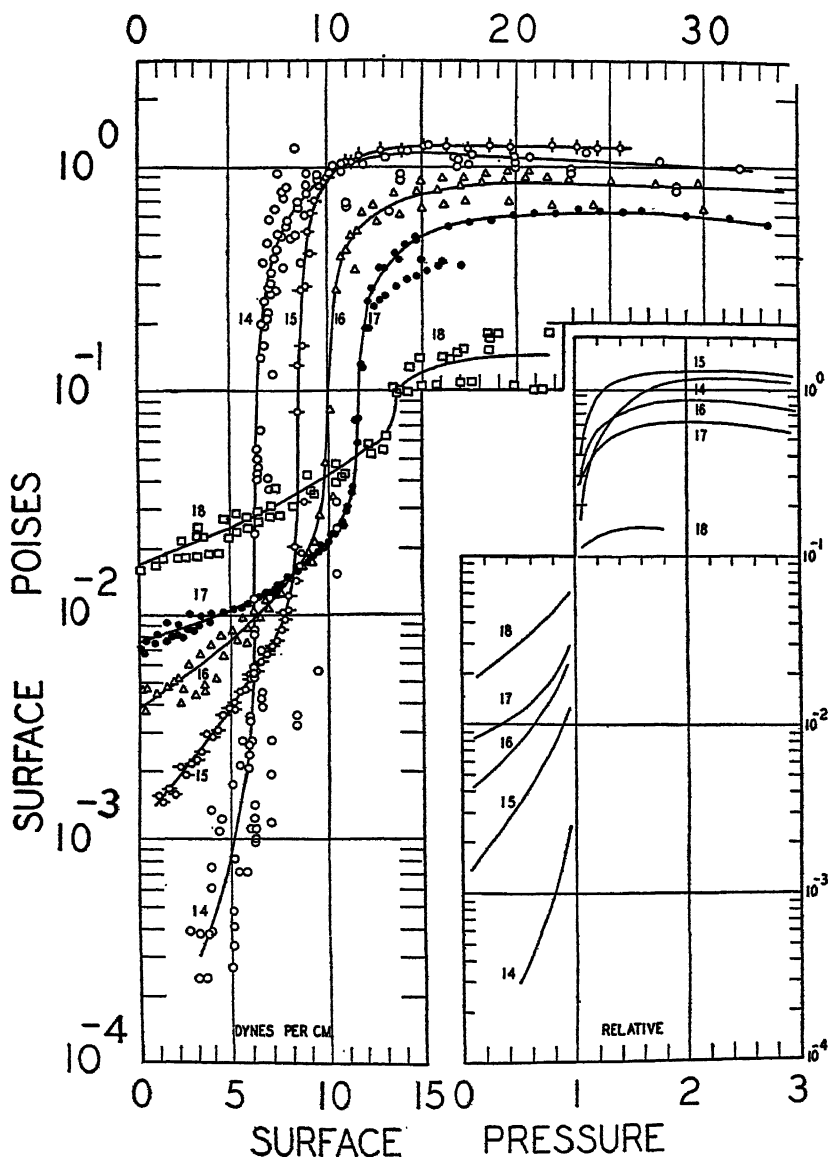


FIG. 9. Viscosity-pressure relations of normal long-chain alcohols. Ordinate, logarithm of surface viscosity; abscissa, surface pressure. In the insert the surface pressure scale is in multiples of the mean kink-point pressure.

viscosities are much higher if the number of carbon atoms is the same (figure 9). Since the pressure of solidification is much lower than with the alcohols, the linear part of the π - $\log \eta$ curve is very short. Thus

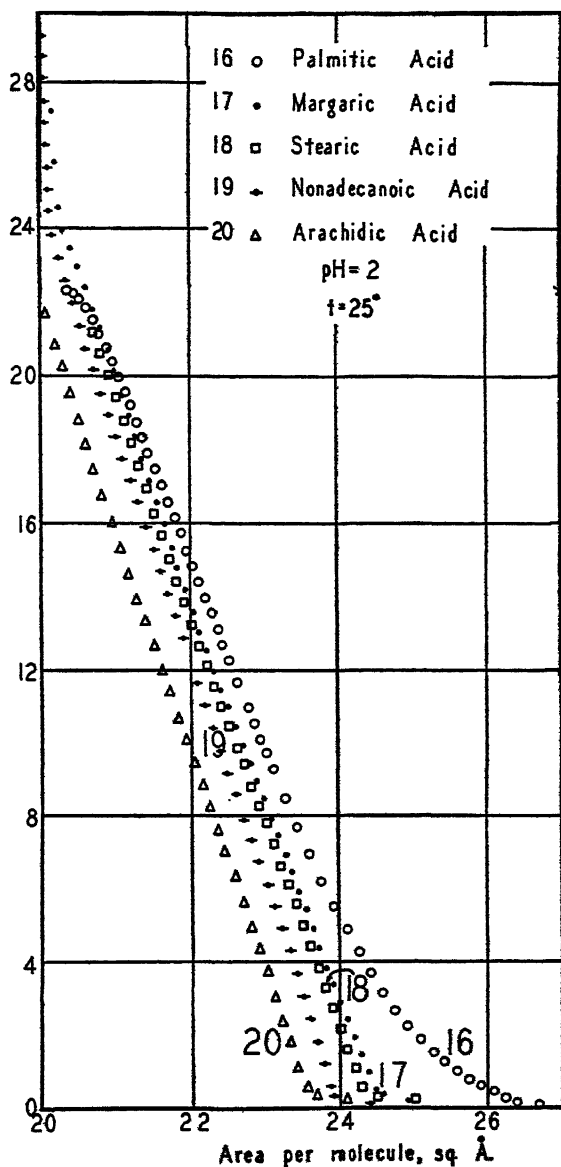


FIG. 10. Pressure-area relations of liquid condensed films of normal long-paraffin-chain acids

with the seventeen-carbon-atom alcohol the relation is linear up to only about a pressure of 7 dynes cm^{-1} , since above this the viscosity is increased abnormally by the approach of the pressure to that of solidification (12 dynes cm^{-1}).

The viscosity of the solid alcohol or acid *decreases* rapidly with the length of the hydrocarbon chain. As a result of this it is found that with long chains the viscosity of the solid is only slightly greater than that of

TABLE 4

Limiting areas (at $\tau = 0$) and compressibilities of long-chain acids and alcohols

A. Acids at 25°C.*

CARBON ATOMS PER MOLECULE	ACID	LIMITING AREA (SQ. Å. PER MOLECULE)	SLOPE (SQ. Å. PER DYNE)	COMPRESSIBILITY	
				At $\tau = 0$	At $\tau = 20$
14	Myristic	46.4			
15	Pentadecylic	45.6			
16	Palmitic	26.75			
17	Margaric	24.59	-0.180	-0.0073	-0.0086
18	Stearic	24.41	-0.177	-0.0072	-0.0085
19	Nonadecanoic	24.0	-0.163	-0.0065	-0.0075
20	Arachidic	23.64	-0.159	-0.0067	-0.0078
CARBON ATOMS PER MOLECULE	ACID	CHANGE OF COMPRESSI- BILITY (PER CENT)			
14	Myristic				
15	Pentadecylic				
16	Palmitic				
17	Margaric	17.1			
18	Stearic	16.8			
19	Nonadecanoic	15.0			
20	Arachidic	16.2			

B. Alcohols at 20°C. Pressure-area data for alcohol monolayers

CARBON ATOMS PER MOLE- CULE	ALCOHOL	LIMITING AREA (SQ. Å. PER MOLE- CULE) AT $\tau = 0$		KINK-POINT PRESSURE (DYNES PER CM.)		SLOPE (SQ. Å. PER DYNE)		COMPRESSIBILITY	
		Liquid	Solid			Liquid	Solid	Liquid at $\tau = 0$	Solid at $\tau = 20$
14	Tetradecyl	22.02	21.1	6.2		0.226	0.084	0.0103	
15	Pentadecyl	21.91	21.0	8.2	2.0	0.138	0.030	0.0063	0.0015
16	Hexadecyl	21.85	20.9	10.2	2.0	0.121	0.021	0.0055	0.0010 ₂
17	Heptadecyl	21.76	20.6	11.8	1.6	0.117	0.021	0.0054	0.0010 ₄
18	Octadecyl	21.82	20.6	12.9	1.1	0.106	0.014	0.00485	0.0007 ₆

* At this temperature the fourteen-carbon-atom and fifteen-carbon-atom acids are in the L_1 state, and the acids containing from sixteen to twenty carbon atoms are in the condensed L_2 state.

the liquid, while with short chains it is very much greater. Thus the viscosity of the liquid monolayer tetradecyl alcohol is 10^{-3} poise at $\tau = 5$, but that of the solid is 1 poise at $\tau = 10$ dynes cm.⁻¹, i.e., there is a thou-

sandfold increase in viscosity. Very remarkable results on the pressure-area-temperature and pressure-viscosity-temperature relations of octa-decyl alcohol in the neighborhood of 10°C. have been obtained in this laboratory by L. E. Copeland, but these are too complicated to discuss in this review.

It is usually stated that all normal long-chain paraffin acids occupy the same molecular area in the condensed liquid state, but actually (19), at a low film pressure, the molecular area decreases by about 0.3 \AA^2 for each carbon atom added to the chain (figure 10) (reference 19, page 1186). The limiting areas and compressibilities of certain condensed liquid monolayers are given in table 4. The limiting area is the area obtained at zero pressure by extrapolation of the straight line which gives the pressure-area relation.

TABLE 5

Dependence of the compressibility on the state of the monolayer

Gas. Perfect gas: $\pi\sigma = kT$	
Imperfect gas: minimum of same order as for L_1 films; e.g., 0.04 for ethyl palmitate at 25°C., 34 \AA^2 , and 14 dynes cm^{-1}	
II	Liquid expanded, L_1 : $-\kappa = 2 \text{ to } 7 \times 10^{-2}$
III	Intermediate, I .
	Maximum $-\kappa$ of order of
	2 to 5×10^{-1} for normal long-chain acids
	2.2×10^{-1} for normal long-chain esters
	Minimum same as $-\kappa$ of phase IV = L_2
IV	Liquid condensed, L_2 : $-\kappa = 5 \text{ to } 10 \times 10^{-2}$
V	Solid, S : $-\kappa = 7 \text{ to } 9 \times 10^{-4}$

The compressibility of the L_2 monolayer is of the order of ten times that of the solid, that for L_1 is of the order of six times that for L_2 , and the maximum for the intermediate phase is of the order of eight times that for L_1 , while its minimum compressibility is in the limit equal to that of L_2 . Since the maximum compressibility of the intermediate phase is somewhat dependent upon the rate of compression, the value for any given substance and temperature is more uncertain than for the other phases.

VII. THE COMPRESSIBILITY OF MONOLAYERS

The compressibility of a monolayer is dependent upon the extent to which the film is already contracted by intermolecular forces, but is not always smaller at smaller areas, since it is also dependent on the phase involved.

Table 5 shows that each phase, as L_1 , I , L_2 , etc., is characterized by its own order of compressibility.

Table 6 lists the compressibilities of certain acid, alcohol, and ester monolayers.

TABLE 6
Compressibility (κ) of monolayers in different states

NUMBER OF CARBON ATOMS	SUBSTANCE	MOLECULAR AREA (σ)	PRESSURE (π)	TEMPERATURE, $^{\circ}\text{C}$.	COMPRESSIBILITY ($-\kappa$)
Liquid (L_1) state expanded					
13	Tridecylic acid	26-31		12.5	0.019-0.027
	Tridecylic acid	32-41		12.5	0.026-0.06
14	Myristic acid	31-37.5		16.6	0.02-0.04
	Myristic acid	37.5-43.5		16.6	0.04-0.07
15	Pentadecylic acid	31.5-39.5		25.0	0.025-0.035
	Pentadecylic acid	39.5-44		25.0	0.035-0.045
57	Triolein	103	8-16	7	0.03
	Triolein	126	0-8	7	0.06*
18	Ethyl palmitate (gas)	40	8.5	25	0.04
18	Octadecanenitrile	35.5-45	0.25-6.5	25.8	0.03-0.06
Intermediate (I): nitriles					
18	Octadecanenitrile	35.5-27	6.5-16	25.8	0.02-0.06
Intermediate state: acids					
14	Myristic acid	35	3	9.5	0.25
15	Pentadecylic acid	40		17.9	0.35
Intermediate state: esters					
18	Ethyl palmitate	34.7	9.3	25	0.53
19	Ethyl margarate	47	3.5	25	1.2
	Ethyl margarate	58	2.1	15.2	2.2
Liquid condensed (L_2): acids					
15	Pentadecylic acid	21-23.2		27.5	0.005-0.027
16	Palmitic acid	21-23.7		25	0.008-0.01
17	Margaric acid	21.6-25		25	0.007-0.0085
18	Stearic acid	21-24		25	0.0063-0.0095
19	Nonadecanoic acid	20.4-23.6		25	0.0063-0.0075
20	Arachidic acid	20.6-24.2		25	0.0065-0.0084
Liquid condensed (L_2): alcohols					
18	Octadecanol	22.6-20.54	1.5-14	25.12	0.0055-0.008
		21.8-20.4	1.5-12.4	9.90	0.004-0.016
		21.8-19.78	1.5-14.9	7.10	0.0055-0.008
19	Nonadecanol	22.7-20.7	1.5-15.3	21.73	0.0055-0.008
		22.6-20.5	1.5-14.7	14.83	0.0055-0.008
		22.3-20.0	1.5-17.4	10.35	0.0047-0.008
20	Eicosanol	22.3-20.33	1.5-16.2	24.76	0.004-0.009
		22.3-20.3	1.5-15.7	22.43	0.004-0.016
		21.9-19.92	1.5-19.3	18.34	0.004-0.009

TABLE 6—*Concluded*
Compressibility of monolayers in highly condensed states

NUMBER OF CARBON ATOMS	SUBSTANCE	MOLECULAR AREA (σ)	PRESSURE (π)	TEMPERATURE, $t^\circ\text{C}$.	COMPRESSIBILITY ($-\kappa$)
------------------------	-----------	-----------------------------	--------------------	----------------------------------	-------------------------------

LS state: alcohols

(Compressibility of a solid state, but viscosity ranging from much less than for the L_2 state to the high values of the solid or S state: c = collapse)

18	Octadecanol	20.54- c	14.05- c	25.12	0.0014-0.0017
		20.2- c	12.37- c	9.90	0.0007-0.0009
19	Nonadecanol	20.71- c	15.27- c	21.73	0.0008-0.0009
		20.36- c	14.7- c	14.83	0.0005-0.0009
20	Eicosanol	20.33- c	16.20- c	24.76	0.0005-0.0009
		20.24- c	15.70- c	22.48	0.0008-0.0011

S state (usually designated as the solid (two-dimensional) state)

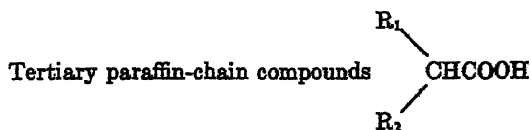
Alcohols

18	Octadecanol	19.78- c	14.90- c	7.10	0.0006-0.0008
19	Nonadecanol	19.97- c	17.40- c	10.35	0.0007-0.0009
20	Eicosanol	19.92- c	19.30- c	18.34	0.0007-0.0010

Acids, esters

17	Margaric acid	20	30	25	0.0008
18	Stearic acid	20	40	22.5	0.0009
18	Octadecyl alcohol	20.3	20	20	0.0007
18	Calcium stearate	20	0.5	22.7	0.0008
19	Ethyl margarate	20.5	19.5	20	0.0020

Section 2: Effect of a divided chain



	R ₁	R ₂				
15	1 C	14 C	30	18	18	0.013
15	7 C	8 C	62	14	18	0.020
16	8 C	8 C	55	15	18	0.017
12	6 C	6 C	58	7.5	18	0.026

Section 3: Effect of orientation: molecules which lie flat on the surface

40 to 1480	ω -Hydroxydecanoic acid polymers	250 to 10,000	1.5-6	20	0.046-0.058
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VIII. THERMODYNAMICS OF SPREADING AND EXTENSION OF FILMS

The expansion of a film may be brought about by two different processes which involve quite different amounts of energy: (1) Spreading may be defined as the process in which the area (σ_f) of a film, either duplex or monomolecular, increases by an amount $\Delta\sigma_f$, while the area of the surface of the subphase (e.g., water, mercury, etc.) decreases by $\Delta\sigma_f$. (2) Extension is the process in which the area of the film and of the subphase remains constant.

The maximum work (δW) done by a system when its area (σ) is increased or decreased is

$$\delta W = -\gamma d\sigma + p dv \quad (10)$$

where γ is the surface tension. The zeta function of Gibbs is defined by him as

$$F = E - ST + pv \quad (11)$$

The Helmholtz free energy is defined as

$$A = E - ST \quad (12)$$

so

$$F = A + pv \quad (13)$$

If a process is reversible and isothermal, $T\Delta S = Q$ is the heat absorbed, and

$$dA = -dW \quad (14)$$

$$dF = -dW + p dv + v dp \quad (15)$$

From equations 10 and 15

$$dF = \gamma d\sigma + v dp \quad (16)$$

so if p is constant

$$(\partial F / \partial \sigma)_{p,T} = \gamma \quad (17)$$

and

$$(\partial F / \partial \sigma)_{p,T} d\sigma = \gamma d\sigma \quad (18)$$

For a saturated surface $\gamma = f(p, T)$ only, so

$$\Delta F = \gamma \Delta \sigma \quad (19)$$

If a liquid b is to spread on the surface of a liquid a , $(\partial F / \partial \sigma)_{p,T}$ should be negative for the summation of all of the terms involved. Thus if b

spreads on a to form a duplex film, then the condition for this type of spreading to occur is

$$\begin{aligned} dF &= \left(\frac{\partial F}{\partial \sigma_b} \right)_{p,T} d\sigma_b + \left(\frac{\partial F}{\partial \sigma_{ab}} \right)_{p,T} d\sigma_{ab} + \left(\frac{\partial F}{\partial \sigma_a} \right)_{p,T} d\sigma_a \\ &= \gamma_b d\sigma_b + \gamma_{ab} d\sigma_{ab} + \gamma_a d\sigma_a < 0 \quad (20) \end{aligned}$$

For a film of appreciable thickness the effects of gravitation must also be considered, but they may be neglected here. If it is assumed that

$$d\sigma_b = d\sigma_{ab} = -d\sigma_a \quad (21)$$

which is practically valid for a very thin layer, then

$$\frac{dF}{d\sigma} = \gamma_b + \gamma_{ab} - \gamma_a \quad (22)$$

Let

$$-(\partial F / \partial \sigma)_{p,T} = S_{b/a} \quad (23)$$

be defined as the spreading coefficient; then

$$-(\partial F / \partial \sigma)_{p,T} = S_{b/a} = \gamma_a - (\gamma_b + \gamma_{ab}) \quad (24)$$

If the liquids are pure and the surfaces clean, then $S_{b/a}$ may be designated as the initial spreading coefficient, and spreading will occur as a duplex film if this is positive.

If b is saturated with a , but a is entirely free from b , then $S_{b'/a}$ is the semi-initial spreading coefficient, while $S_{b'/a'}$ is the final spreading coefficient. The prime in b' indicates that the liquid b is saturated with the other liquid (a).

From equations 3, 4, and 5,

$$S_{b/a} = W_A - W_{e_b} \quad (25)$$

The pressure π_f of a non-duplex film or monolayer is defined by the equation:

$$\pi_f = \gamma_a - \gamma_f \quad (26)$$

where γ_f is the free surface energy of a when covered by the film f .

Now the final spreading coefficient $S_{b'/a'}$ indicates that the lens of b' is in equilibrium with the film f , so γ_f may be written γ_e to specify equilibrium. Thus

$$S_{b'/a'} = \gamma_e - (\gamma_{b'} + \gamma_{a'b'}) \quad (27)$$

The semi-initial coefficient is

$$S_{b/a} = \gamma_a - (\gamma_{b'} + \gamma_{a'b'}) \quad (28)$$

and equation 28 minus equation 27 gives

$$S_{b/a} - S_{b'/a'} = \pi_e \quad (29)$$

or, the film pressure is the difference between the semi-initial and the final spreading coefficients.

Since the final coefficient is always negative

$$\pi_e > S_{b/a} \quad (30)$$

or, the equilibrium film pressure of the monolayer is always greater than the semi-initial spreading coefficient.

IX. HEATS OF SPREADING AND EXTENSION

The heat content (H) is defined by

$$H = E + pv \quad (31)$$

or

$$dH = dE + p dv + v dp \quad (32)$$

and from equation 2

$$H = F - ST \quad (33)$$

or

$$dH = dF + T dS + S dT \quad (34)$$

In the treatment which follows, energy values designated by small letters refer to the change of energy for unit area. Thus in

$$h = (\partial H / \partial \sigma)_T$$

the h designates the increase of heat content of a film of very large area for unit increment of area.

Consider a trough filled with a body of water with a plane surface partly covered with a monolayer. The area of the film, together with that of the subphase, may be increased by 1 sq. cm. by the use of some mechanical device while the area of the water surface is kept constant. Thus a ring of suitable dimensions, already lying in the surface, may be pulled upward until there is a unit increase in the area of the film. If the

film area is thus increased by $d\sigma_f$, instead of unity, the equation for the process which may be designated as extension (e), may be written

$$dF_e = (\partial F / \partial \sigma_f)_{T, \sigma_w} d\sigma_f = \gamma d\sigma_f \quad (35)$$

or

$$\Delta F(\Delta \sigma = 1 \text{ cm.}^2) = (\partial F / \partial \sigma_f)_{T, \sigma_w} = f_e \quad (36)$$

which gives the free energy of extension of the film. The entropy of extension is

$$s_e = (\partial S / \partial \sigma_f)_{T, \sigma_w} = -(\partial \gamma_f / \partial T)_{\sigma_f} \quad (37)$$

The increment of heat content is given by

$$h_e = (\partial H / \partial \sigma_f)_{T, \sigma_w} = \left[\frac{\partial(\gamma_f / T)}{\partial(1/T)} \right]_{\sigma_f} \quad (38)$$

and the heat absorbed (q) by

$$q_e = T(\partial S / \partial \sigma_f)_{T, \sigma_w} = -T(\partial \gamma_f / \partial T)_{\sigma_f} = Ts_e \quad (39)$$

Similar equations may be written for the energy of spreading of a film. This process (s) may be assumed to occur as follows: The surface of the water in the trough is separated into two parts by a movable barrier. On one side of the barrier the surface is covered by the film, on the other side the water surface is clean. Let the barrier be moved in such a way that the area of the film is increased by 1 sq. cm., and that of the water is decreased by this amount.

The equations for spreading are:

$$\begin{aligned} dF_s &= (\partial F / \partial \sigma_f)_{T, \Sigma} d\sigma_f - (\partial F / \partial \sigma_w)_{T, \Sigma} d\sigma_w \\ &= -(\gamma_w - \gamma_f) d\sigma_f = -\pi d\sigma_f \end{aligned} \quad (40)$$

where $\Sigma = \sigma_w + \sigma_f$.

The increase in entropy which accompanies the spreading of the film over the water is, from equation 16,

$$S_s = (\partial S / \partial \sigma_f)_{T, \Sigma} = -(\partial^2 F / \partial T \partial \sigma_f)_{T, \Sigma} = -(\partial \pi / \partial T)_{\sigma_f} \quad (41)$$

Figure 11 shows the variation of π with T for two long-chain acids. The heat absorbed is

$$q_s = Ts_s \quad (42)$$

and the increase in heat content

$$h_s = (\partial H / \partial \sigma_f)_{T, \Sigma} = - \left[\frac{\partial(\pi / T)}{\partial(1/T)} \right]_{\sigma_f, \Sigma} \quad (43)$$

Equations 15 to 18, which give the energy and entropy of extension for the films, are also applicable to pure liquids. Thus, using equation 17, the increase in the heat content when the surface area of water is increased is found as $h = 116$ ergs per cm.^{-2} at 20°C .

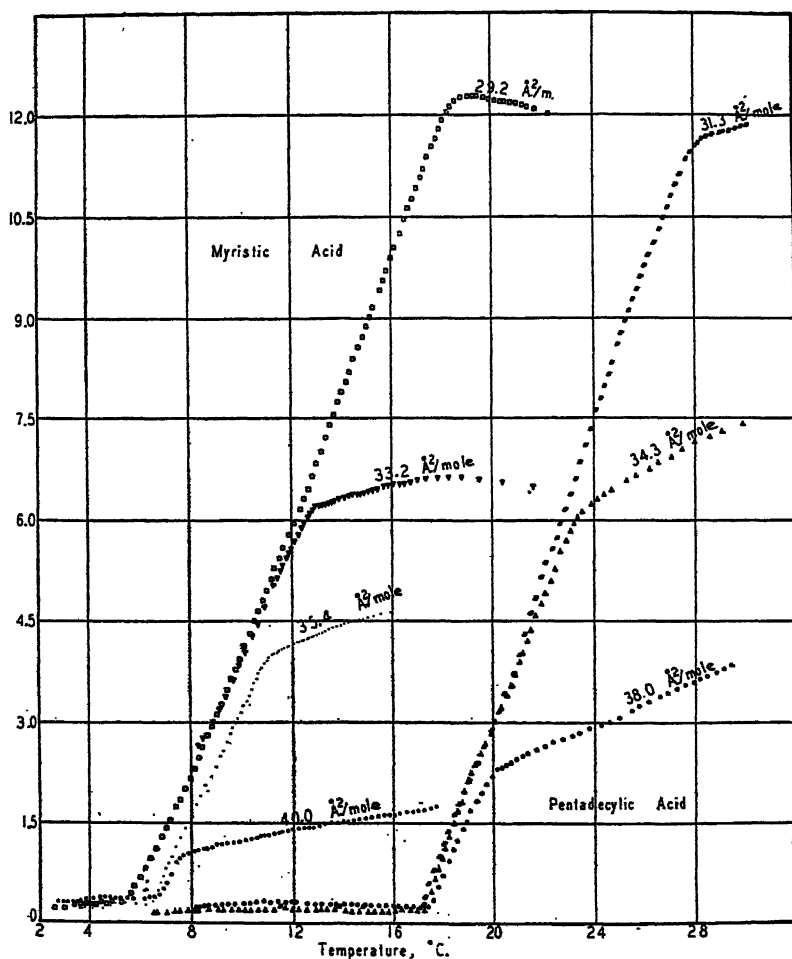


FIG. 11. Variation of the film pressure of intermediate and expanded films with temperature. The slope $(\partial\pi/\partial T)_{p,\sigma}$ is the entropy of the film.

In equation 26 π is defined as

$$\gamma_w - \gamma_f \quad (44)$$

and it can be shown that, in equation 16,

$$s_e = s_s + s_w \quad (45)$$

and in equation 17,

$$h_e = h_s + h_w \quad (46)$$

The energy changes of the greatest interest are those associated with the isothermal expansion (or compression) of a film between any molal area, σ_1 , and any other molal area, σ_2 . The heat content and heat absorbed may be calculated for either the spreading or the extension of the film. For one mole of material the increase of heat content is

$$\Delta H_m = \int_{\sigma_1}^{\sigma_2} (\partial H / \partial \sigma)_T d\sigma_m = \int_{\sigma_1}^{\sigma_2} h d\sigma_m \quad (47)$$

and the heat absorbed:

$$Q_m = T \int_{\sigma_1}^{\sigma_2} (\partial S / \partial \sigma)_T d\sigma_m = T \int_{\sigma_1}^{\sigma_2} s d\sigma_m \quad (48)$$

where ΔH_m and h of equation 23 and Q_m and s of equation 24 stand for $(\Delta H_m)_e$, h_e , $(Q_m)_e$, and s_e when the process is one of extension, and for $(\Delta H_m)_s$, $(Q_m)_s$, and s_s when the process is one of spreading.

The heat absorbed may readily be calculated from ΔH and ΔF of any process. For spreading:

$$(Q_m)_s = (\Delta H_m)_s - (\Delta F_m)_s = (\Delta H_m)_s + \int_{\sigma_1}^{\sigma_2} \pi d\sigma_m \quad (49)$$

For extension:

$$(Q_m)_e = (\Delta H_m)_e - (\Delta F_m)_e = (\Delta H_m)_e - \int_{\sigma_1}^{\sigma_2} \gamma d\sigma_m \quad (50)$$

X. ENERGY MAGNITUDES ASSOCIATED WITH THE SPREADING OR EXTENSION OF MONOLAYERS

The expansion of the surface of a pure liquid, or of a film-covered surface, may be considered to occur by the process which has been considered as *extension*. The energy of extension per square centimeter of surface formed may be considered in relation to any one of the following energy quantities: f , the increase of free energy; h , the increase of enthalpy or ϵ , the increase of internal energy, which is equal to h within the limits of error of their determination; q , the heat absorbed; and s , the entropy.

The value of h_e or ϵ_e for the extension of a water surface at 20°C. is 116 ergs cm.⁻², but for oils the values are small, and in the same units are: propane, 34; heptane, 50; octane, 50; propyl alcohol, 48; butyl alcohol, 48.5; heptylic acid, 52.5; octyl alcohol, 50.5.

It might be thought, in view of the above facts, that the increase in potential energy (ϵ_e) for the extension of a monomolecular film of oil and

the water upon which it lies, would be of the order of the above values, particularly if the principle of independent surface action is considered in this connection. However, this is very far from what is obtained by the application of the thermodynamic equations to the experimental data. This gives values of the increase of internal energy, in ergs per square centimeter of film produced by extension, as low as *zero* for long-chain alcohols in certain peculiar regions, 120 for condensed films of long-chain acids, 400 for some of their intermediate films, and as high as 900 for films, usually classed as solid monolayers, of the normal long-paraffin-chain alcohols.

A few representative values, which do not, however, include the minimum and maximum cited above, are given in table 7. This table shows that high values of the energy of extension or of spreading found for certain two-dimensional phases (intermediate and solid) are due to the abnormally large entropy of the monolayer, since the free energy of the oil film is in each case less than that of water. With solid alcohol monolayers the entropy (s_s) has been found to be as large as $2.6 \text{ ergs cm.}^{-2} \text{ deg.}^{-1}$. In the case of long-chain acids, the highest value of the entropy is found in the intermediate state. The highest values yet found are those for the solid long-chain alcohol monolayers, but values for the intermediate phase of the alcohols have not been obtained, on account of the high temperature required.

Let us consider the heat (q_s) absorbed by the spreading at 20°C. of a pentadecylic acid monolayer over 1 sq. cm. of the surface of water. On account of the instability of the solid film q_s is not known, but from the high values obtained for alcohols it may be expected to have a value of about 200 ergs. At E (figure 4) the solid monolayer melts with a *zero* heat of fusion, so the phase change is of the second order. The liquid condensed film thus formed absorbs practically no heat as it expands by spreading. At D there is a change, presumably of the third order, to the intermediate film for which q_s is also zero adjacent to D but rises rapidly to a nearly constant value of $280 \text{ ergs cm.}^{-2}$. At C there is a phase transformation to liquid expanded film, and the heat of spreading drops quickly to about 50 ergs cm.^{-2} .

Let us suppose that we begin with a monolayer of pentadecylic acid at 21.7°C. and a molecular area of 20.0 \AA.^2 and allow it to expand by spreading to 20.5 \AA.^2 . The heat absorbed is of the order of 0.15 kg.-cal. At E the monolayer melts without the absorption of heat, and it expands to (D) 20.8 \AA.^2 without absorbing any heat. The heat absorbed increases rapidly to a maximum per unit area, and the total heat absorbed in expanding by spreading through the whole intermediate state is 6.4 kg.-cal. At an area of 35.5 \AA.^2 per molecule the film changes to the liquid expanded state

TABLE 7

A. Energy (ϵ) and entropy (s) of extension and of spreading of monolayers on water
 Energy in ergs cm.⁻²; entropy in ergs cm.⁻² degree⁻¹

	f	ϵ	$q = Ts$	s
Liquid monolayers: pentadecylic acid	Expanded film at 22°C. and $\sigma = 38 \text{ Å}^2$.			
	A. Clean water	72.46	116 ₄	44.00
	B. Film (extension)	69.80	161	91.6
	C = B - A (spreading)	-2.64	45	47.6
	Intermediate film at 20°C. and $\sigma = 34.3 \text{ Å}^2$.			
	A. Clean water	72.75	116	43.25
	B. Film (extension)	69.75	394	324
	C = B - A (spreading)	-3.0	278	281
	Intermediate film at 18°C. and $\sigma = 38 \text{ Å}^2$.			
	A. Clean water	73.05	115.5	42.49
	B. Film (extension)	72.32	367	295
	C = B - A (spreading)	-0.73	252	253
Liquid monolayers: hexadecyl alcohol at $\sigma = 20.0 \text{ Å}^2$.	Condensed liquid film at 9°C. $\tau = 7.7$ dynes per cm.			
	A. Clean water	74.37	115.5	41.17
	B. Film (extension)	66.7	166	99.6
	C = B - A (spreading)	-7.7	50.7*	58.4
Phase of low compressi- bility, usually desig- nated as "solid"	At 16°C. $\tau = 13.8$ dynes per cm.			
	A. Clean water	73.34	116.1	42.77
	B. Film (extension)	59.5	508	449
	C = B - A (spreading)	-13.8	392	406

B. Effect of pressure on the energy relations of octadecanol at $\sigma = 19.96 \text{ Å}^2$.

Phase	τ	t in °C.	ϵ_s	q_s	s_s
L_2	16	7.34	264	280	1.00
S	17	7.88	1528	1545	5.5
	18	8.06	1630	1648	5.9
	19	8.28	1062	1081	3.8
	20	8.57	760	780	2.8
	21	8.92	710	731	2.6
	22	9.29	617	639	2.3
	23	9.68	780	803	2.8
	24	10.05	845	869	3.1
LS	25	10.38	602	627	2.2
	29	12.95	385	414	1.45

* Values for the increase in internal energy on spreading (ϵ_s) of the condensed liquid (L_2) monolayer of a long-chain alcohol have been found in the range from 36 to 200 ergs cm.⁻², but with a long-chain normal acid the values lie in the vicinity of zero, so that the energy of extension of films of the acids is about 116 ergs cm.⁻², i.e., equal to the energy of extension of the water surface without the film.

and by further expansion to 44 \AA^2 absorbs 2.1 kg.-cal if the process is spreading. The total heat absorbed in expansion from 20 to 44 \AA^2 per molecule is 8.65 kg.-cal. none of which is used in melting the solid, and most of which is used in expansion in the intermediate state.

Table 8, taken from the work of Harkins, Young, and Boyd (14), gives the increase in enthalpy (ΔH) and the heat absorbed in the spreading and in the extension of a monolayer of pentadecylic acid. As the temperature rises, the amount of heat absorbed in the intermediate state decreases, while that in the expanded state increases. The total increase of enthalpy (ΔH), or of internal energy (ΔE), in passing through both the intermediate and the expanded state seems, if the process is one of extension, to decrease slowly as the temperature rises.

TABLE 8

Heat absorbed (Q_m) and increase of heat content (ΔH_m) for the spreading and extension of a pentadecylic acid film in the intermediate phase (EC) and the liquid expanded phase (CB)

Energy in kg.-cal. per mole; area (σ) in \AA^2 per molecule; energy values calculated from those for ϵ_s in table 7

INTERMEDIATE PHASE							LIQUID EXPANDED PHASE						
σ_1	σ_2	t°	H_m		Q_m		σ_1	σ_2	ΔH_m		ΔQ_m		
			Spreading	Extension	Spreading	Extension			Spreading	Extension	Spreading	Extension	
21.5	42.0	19.0	8.3	11.8	$\Delta H_m - 0.080$	9.6	42.0	43	Very small since $\Delta\sigma$ very small				
20.8	35.5	21.7	6.4	8.9	$\Delta H_m - 0.160$	7.4	35.5	44	0.7	2.1	$\Delta H_m - 0.025$	1.2	
20.5	33.5	25.0	5.4	7.6	$\Delta H_m - 0.0190$	6.2	33.5	45	1.0	3.1	$\Delta H_m - 0.054$	1.8	
20.5	31.5	27.5	4.6	6.5	$\Delta H_m - 0.0195$	4.9	31.5	46	1.3	3.7	$\Delta H_m - 0.097$	2.2	

With esters the maximum value of h_s or q_s in the intermediate state is only about two-thirds that for the acids, but since the intermediate state persists to a much higher area than with the acids, the integral heat (ΔH_m) has about the same magnitude.

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ULTRAVIOLET ABSORPTION SPECTRA OF FATTY ACIDS AND THEIR APPLICATION TO CHEMICAL PROBLEMS¹

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I. INTRODUCTION

The relation of spectral characteristics to the structure of organic compounds was first pointed out by Graebe and Liebermann in 1868 (37). Witt, in 1876, recognized the existence of special structures responsible for specific absorption of light and named them chromophores. According to the earliest theories, double bonds were essential to chromophores and that view is held today.² The simplest and most common of these are ethylenic and carbonyl groups. The position and intensity of absorption bands depend upon the number of such groups, their position with respect to each other (conjugated or unconjugated), and the nature of the substituent groups attached to the unsaturated carbon atoms.

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² Infrared absorption is not being considered here.

Absorption spectroscopy advanced so rapidly that by 1918 Watson's (37) book on *Colour in Relation to Chemical Constitution* was adopted as a reference text by organic chemists. Quantitative spectrophotometry is now rapidly displacing ordinary colorimetry as an analytical tool. However, the early work was confined to the visible and near ultraviolet by the limitation of light sources, spectroscopes, and recording devices. Gradually, reliable measurements are being extended toward the Schumann region through those wave lengths at which the unsaturated fatty acids absorb. In 1918 Watson stated that unsaturated aliphatic hydrocarbons do not show selective absorption in the ultraviolet and cited this as an exception to the general rule that unsaturation is the cause of such absorption. Since that time it has been repeatedly demonstrated that such compounds do absorb strongly in the far ultraviolet, and it is in this region of the spectrum that all of the fatty acids must be studied.

In this laboratory both the photographic and the photoelectric instruments have been used, essentially as recommended by Henri (18), Hogness *et al.* (20), and Harrison *et al.* (16). Modifications in methods now used in our laboratory will be described in a future publication.

The solvent used has an effect on the absorption bands and should be mentioned. This is especially noticeable in the case of salts of the fatty acids dissolved in water, as compared with the acids or esters in organic solvents (25).

The absorption values given in this paper are molecular extinction coefficients (ϵ), absorption coefficients ($E_{1\text{ cm.}}^{1\%}$), or specific absorption coefficients (k). All values are calculated from Lambert's and Beer's law,

$$\log_{10} \frac{I_0}{I} = \epsilon cl$$

ϵ is obtained when the concentration is expressed in moles per liter. For $E_{1\text{ cm.}}^{1\%}$ and k , concentrations are expressed as grams per 100 cc. and grams per liter, respectively. All values are calculated to a cell thickness of 1 cm.

Below 2300 Å. the errors increase and many curves are semi-quantitative. With substances having very low absorption, impurities of high absorption may mask the true bands. Substances with strong bands must be so diluted that they are likely to be oxidized or otherwise changed in the solvent. Carr and Walker (9) point out that the absorption curve of 3-heptene is markedly affected by the presence of so-called peroxides which form readily in most olefins.

II. QUANTITATIVE ABSORPTION SPECTRA

The saturated hydrocarbons, alcohols, and ethers are so transparent that, when pure, they are excellent solvents for measurements at 2000 Å.

or above. With the introduction of a carbonyl group, specific absorption bands appear, as shown in figure 1 (19). Curves for other aldehydes, ketones, and acids have been collected by Henri in the *International Critical Tables* (19). Ketones and aldehydes absorb in a region at which the carboxylic acids are transparent. Comparisons of various carboxylic acids are given in figure 2.

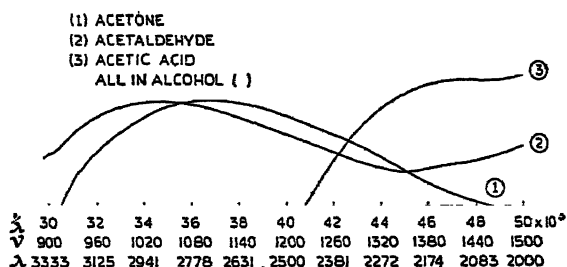


Fig. 1. Absorption coefficients of acetone, acetaldehyde, and acetic acid (after Henri)

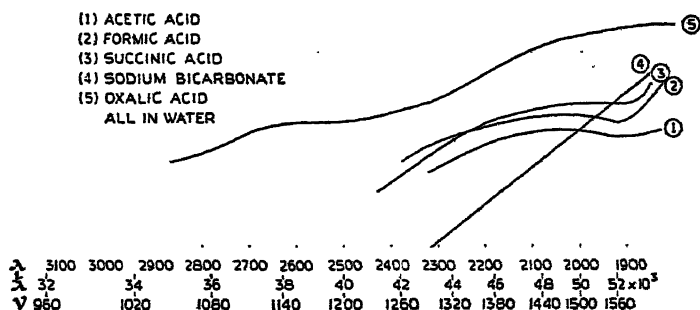


Fig. 2. Ultraviolet absorption spectra of some carboxylic acids (from Ellinger)

Henri (18) established that at the same wave length $\epsilon(\text{succinic}) = 3 \times \epsilon(\text{acetic})$ and $\epsilon(\text{tricarballic}) = 9 \times \epsilon(\text{acetic})$. The very high absorption of oxalic acid is attributed to the conjugated carbonyl groups. Without unsaturation the absorption curves of all fatty acids should closely resemble that of acetic acid. This is indeed the case.

The single ethylenic group of olefins introduces absorption bands, as illustrated in figure 3. The position and intensity of these bands are largely controlled by the number of alkyl groups attached to the unsaturated carbon atoms and are only slightly affected by the number of carbon

atoms in these groups. The quantitative curves for 3-heptene and tetramethylethylene are shown in figure 13.

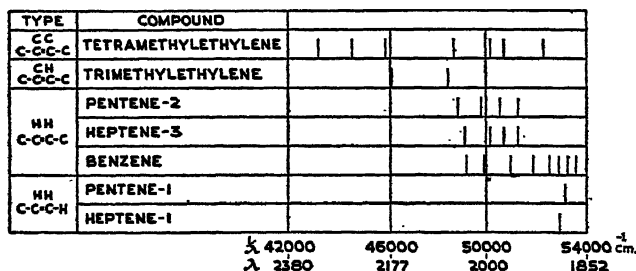


Fig. 3. Absorption bands of olefins in the Schumann ultraviolet (redrawn from Carr and Walker)

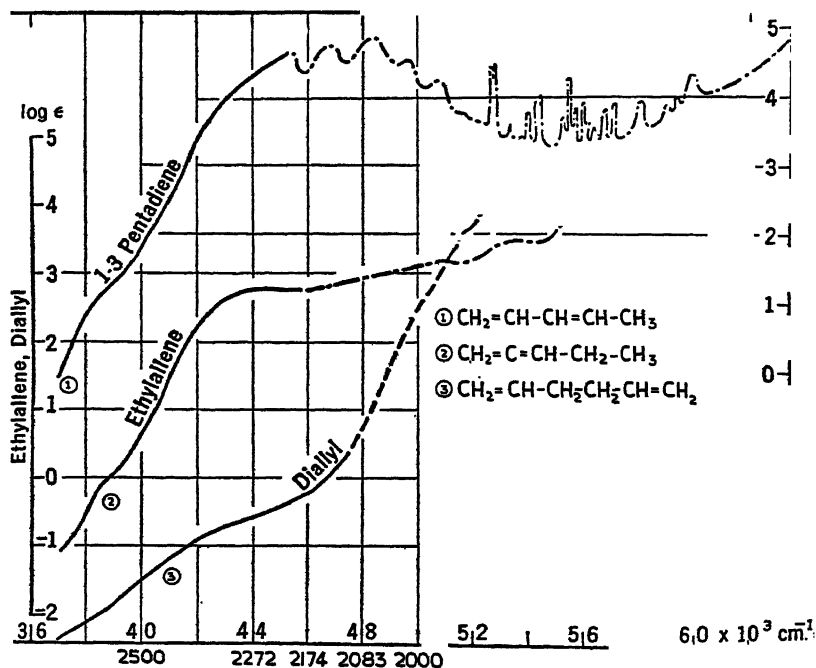


Fig. 4. Absorption curves of hydrocarbon dienes (adapted from Carr and Stücklen). The dotted portions of the curves were run in the vapor phase and give estimated intensities not as quantitative as the measurements on liquids and solutions (solid portions).

The effect of the position of two ethylenic linkages in hydrocarbons is shown in figure 4 (8). When the double bonds are isolated (as in diallyl), there is no mutual interaction and the curve resembles that of 1-pentene

(10). When the double bonds are adjacent (as in ethylallene), the absorption is greatly intensified in the longer wave lengths but is still far less than that of a conjugated system (1,3-pentadiene).

The mixed fatty acids of natural oils exhibit absorption bands due to both the carboxyl and the ethylenic groups. Free fatty acids, glycerides, or other esters may be used more or less interchangeably in these studies. However, if natural oils are used, the absorption by compounds other than the glycerides may predominate. This is the case in many of the curves

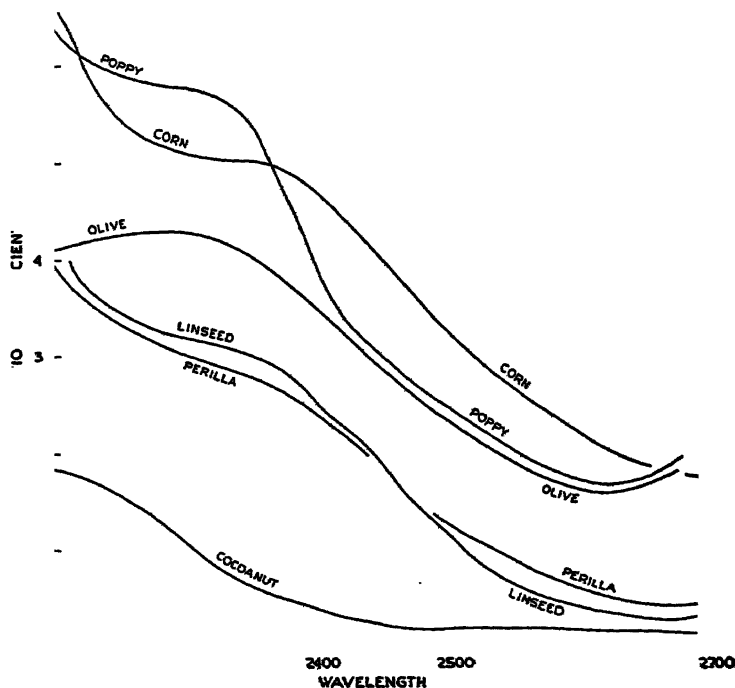


FIG. 5. Absorption spectra of some oils (University of Minnesota.)

in the literature, especially at wave lengths longer than 2300 Å. It is clear from figures 5 (unpublished data from our laboratory) and 6 (5) that, although the highly unsaturated oils absorb more strongly than the saturated oils at wave lengths longer than 2250 Å., there is no direct relationship between the iodine number and the degree of absorption. In natural oils and impure preparations the bands at the longer wave lengths are apparently due to substances other than pure unsaturated fatty acids. Hulst (21) removed the impurities by absorption on bleaching earth (figure 7). He distinguishes between "chemically pure" and "optically pure"

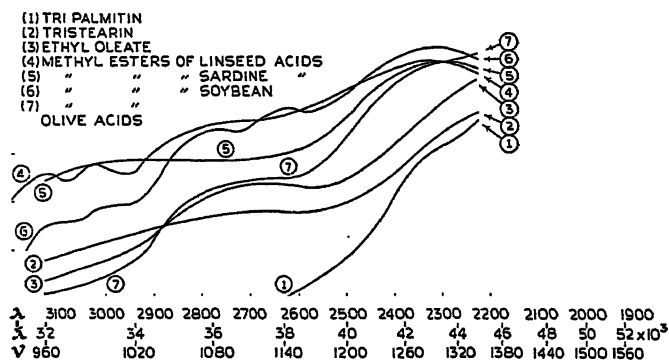


Fig. 6. Absorption spectra of natural fatty acid esters (drawn from data of Bradley and Richardson).

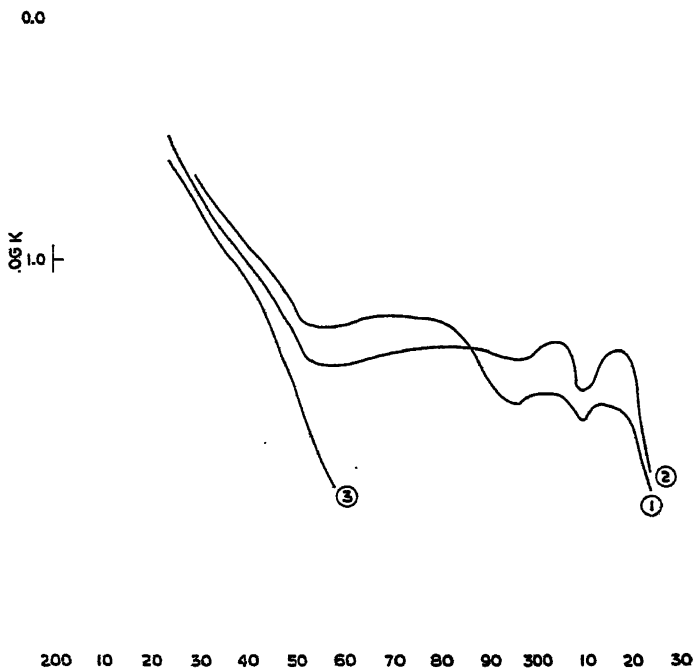


Fig. 7. Absorption spectra of (1) distilled ethyl linolenate, (2) ethyl linolenate after treatment with 10 per cent of bleaching earth, (3) ethyl linolenate after treatment with 100 per cent of bleaching earth (redrawn from Hulst).

ethyl oleate. Extensive spectrographic data for natural fats and their mixed fatty acids are given by Gillam *et al.* (15). Additional curves for plant and animal fats will be found in the reviews by Ellinger (14).

Much of the data for fats and oils is of limited value because the measurements do not extend far enough into the ultraviolet. Many curves stop at 2500 Å. The same is true for the purified fatty acids, and this accounts for the limited material available for comparison.

The saturated fatty acids show small differences in position and height of the absorption bands. Bielecki and Henri (3) compared formic, acetic, propionic, butyric, and valeric acids and found a regular shift toward the red with added $-\text{CH}_2-$ groups and a rise in absorption maximum. Henri (18, page 93) states that for the same absorption there is a shift of $\Delta\gamma = 30 \times 10^{12}$. However, Ley and Arends (25) have made an extensive study of some acids and show that this general rule can not be applied to the higher fatty acids (figure 8). The similarity of acetic and palmitic acids in alcohol is shown by the following data:

Log ϵ	0.35	0.60	0.90	1.10	1.30
λ acetic acid	2411	2390	2350	2315	2269
λ palmitic acid	2428	2400	2360	2325	2295

They, too, find a large shift toward the red for butyric acid in water, but from figure 8 it is clear that in alcohol the higher fatty acids do not continue the shift postulated by Henri. Great similarity in the absorption spectra of all the fatty acids in alcohol was also found by Ramart-Lucas *et al.* (34) for acetic, butyric, hexanoic, octanoic, myristic, and palmitic acids between 2200 and 2555 Å. They say that CH_3- and $-\text{CH}_2-$ groups can not be considered chromophores in this region of the spectrum.

The unsaturated fatty acids absorb more strongly than the saturated, since the effects of the ethylenic linkages are added to the carbonyl absorption. The magnitude of these effects depends upon the number and position of double bonds, as shown by Carr and Stücklen for hydrocarbon dienes (figure 4). Figure 9 presents some absorption curves of well-known fatty acids. All the curves are redrawn from Hulst, except curve 9 which is taken from Kaufmann *et al.* (24). Not shown here is the curve for ricinoleic acid, which Hulst found to be so much like that of oleic acid that he concluded that the hydroxyl group had no characteristic influence. The same may be said about licanic (couepic) acid, which is 4-ketoeleostearic acid. Steger and van Loon (36) compared it with β -eleostearic acid and found the curves to be almost identical. From figure 9 it is clear that the unsaturated acids fall into three groups: those with isolated double bonds (unconjugated) and those with the $\text{C}=\text{C}-\text{C}=\text{O}$ or $\text{C}=\text{C}-\text{C}=\text{C}$.

groupings (conjugated). A discussion of the conjugation of ethenoid and carbonyl groups is given by Morten *et al.* (32) in a spectrographic study of keto-enol isomerism. These authors formulate the hypothesis that "the

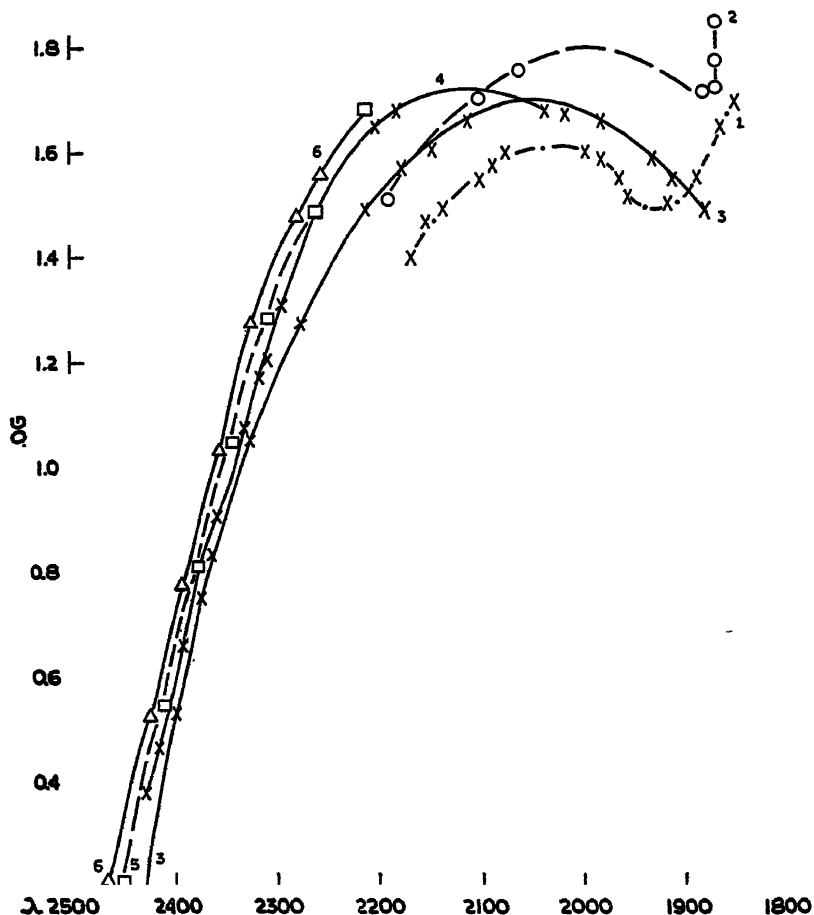


FIG. 8. Absorption spectra of some saturated fatty acids in alcohol, water, and hexane. Curve 1, acetic acid in water (Ley and Arends); curve 2, acetic acid in hexane (Ley and Arends); curve 3, acetic acid in alcohol (Ley and Arends and Henri); curve 4, palmitic acid in alcohol (Ley and Arends); curve 5, butyric acid in alcohol (Henri); curve 6, valeric acid in alcohol (Henri).

different energy levels characteristic of the carbonyl group are the same, or very nearly the same, as those characterizing the ethenoid linkage." It is clear, however, that in a diene like 9,11-linoleic acid the first strong absorption band is shifted much more toward the red. This shift is such

that when measurements do not go below 2200 Å. the maximum absorption of only the conjugated polyenes is observed. The unconjugated acids absorb only slightly more than saturated acids, and the curves for α , β -forms are still rising toward a maximum below 2100 Å. (see figure 12). Figure 10 shows the change in position and intensity of absorption bands with increasing numbers of conjugated double bonds (35). Such smooth curves do not represent the true spectra of conjugated trienes and tetraenes, as shown in figure 9. Kaufmann *et al.* (24) found the number and the intensity of bands in decatetraene to correspond almost exactly with those in parinaric acid, with only slight shifts in position (figure 10a).

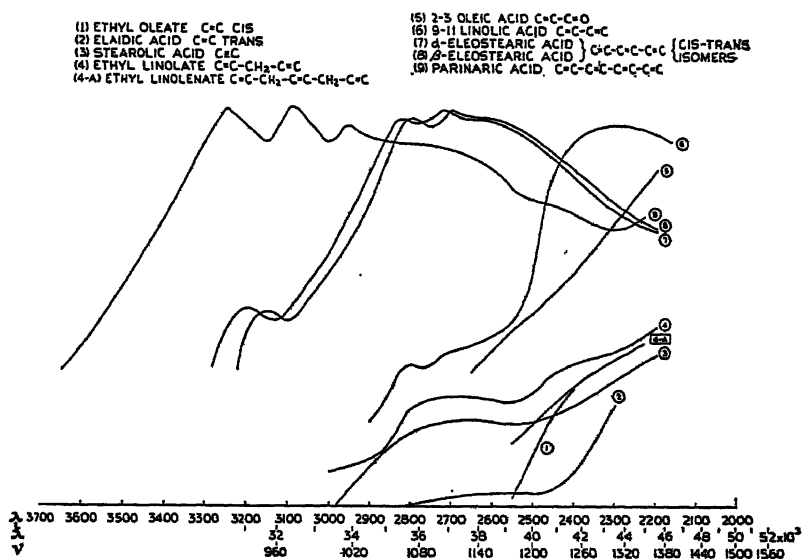


Fig. 9. Absorption spectra of some unsaturated fatty acids (redrawn from Hulst and Kaufmann *et al.*)

Because of possible practical applications, more detailed studies have been made of the natural unsaturated acids, α , β -unsaturated acids, and conjugated dienoic acids. These results are summarized in figures 11, 12, 13, and 14. There is close agreement between our 10,12-linoleic acid and linoleyl alcohol (22) and Hulst's 9,11-linoleic acid. The shift of λ_{\max} may be due to *cis-trans* isomerism (see below). For comparison a curve for dimethylbutadiene is included (19). Except for Hulst's curve, the α , β -unsaturated acids (figure 12) show fairly close agreement, but these values indicate that much more quantitative work is needed. ✓

By the use of the Littrow photographic apparatus the absorption curves for common unsaturated acids have been extended to 2100 Å. in this

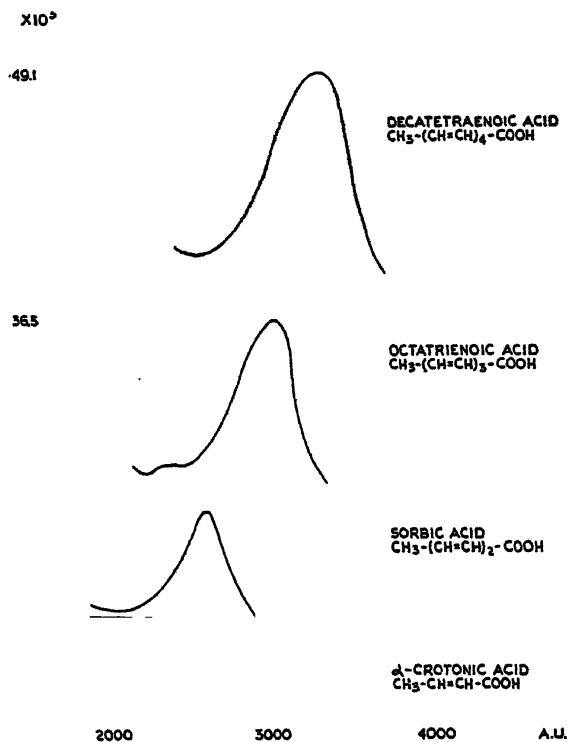


Fig. 10. Absorption spectra of some conjugated acids (redrawn from Smakula)

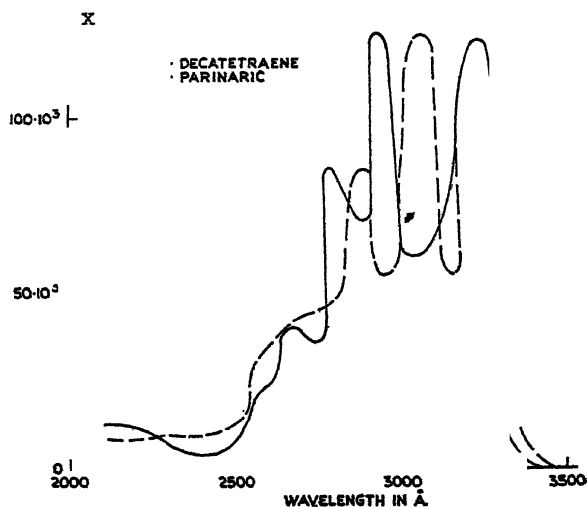


Fig. 10a. Absorption spectra of decatetraene and parinaric acid (from Kaufmann *et al.*)

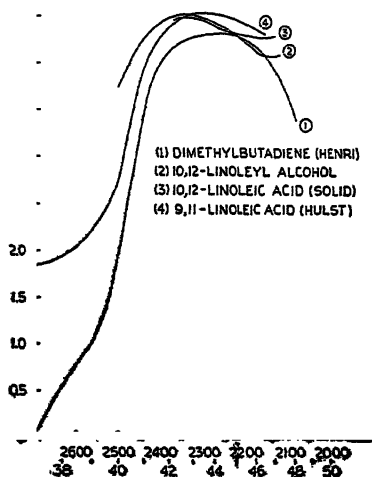


FIG. 11. Absorption spectra of some dienoic compounds. The work on the 10, 12 compounds was done in this laboratory.

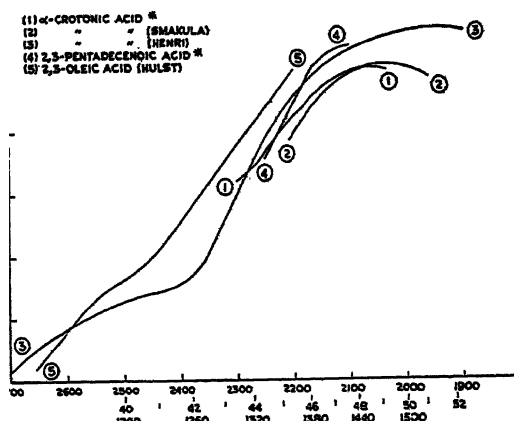


FIG. 12. Absorption spectra of some α,β -unsaturated acids. The starred curves are from this laboratory. The authors are indebted to Dr. Walter Lauer for the sample of 2,3-pentadecenoic acid.

laboratory. Acids of the highest purity available have been used.³ The data are summarized in figure 13. For comparison, the curves of 3-heptene

³ We are indebted to Dr. J. P. Kass for these preparations and to Dr. Richard Barnes and Mr. Irving Rusoff for most of the spectrographic work.

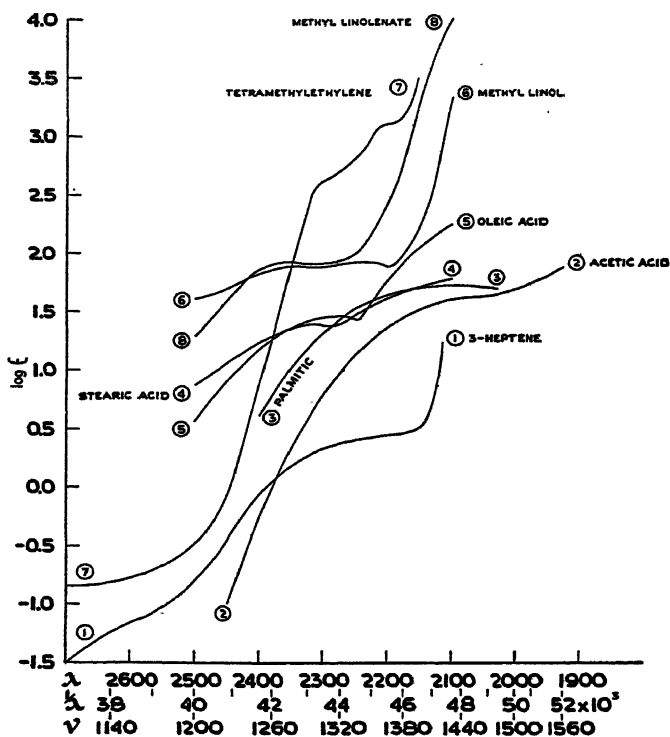


FIG. 13. Absorption spectra of some fatty acids and ethylenic hydrocarbons. Curves 1 and 7 are from Carr and Walker; curve 2 from Henri; curve 3 from Ley and Arends; curves 4, 5, 6, and 8 from this laboratory.

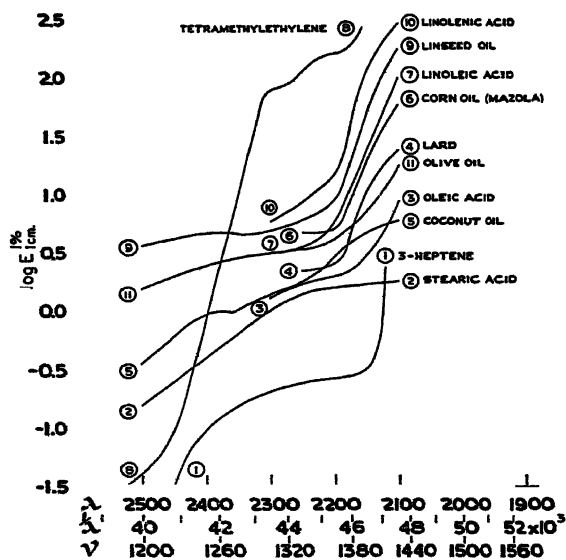


FIG. 14. Absorption spectra of fatty acids and oils, calculated as $E_{1\text{cm}}^{1\%}$.

and tetramethylethylene from Carr and Walker (9) are inserted. Our stearic acid compares well with the palmitic acid of Ley and Arends (25).

All of the unsaturated acids studied superimpose on the carboxylic absorption a band due to the ethylenic groups. In oleic acid the new band starts at slightly longer wave lengths than that of 3-heptene. In the linoleic and linolenic acids this shift continues, showing the influence of additional unconjugated double bonds. These curves are replotted in figure 14 as $\log E_{1\text{cm}}^{1\%}$ for comparison with the absorption of some common oils. It is clear that, except for coconut oil which seems to have had an absorptive impurity, the values fall within the range expected from their known composition. It seems that most of the absorption by oils thus far observed in the far ultraviolet (below 2200 Å.) is due to the known fatty acids. Unfortunately, there is no material in the literature to compare directly with these measurements. The curves of Hulst, of Bradley and Richardson, and of Brode and Tyron (6) do not extend to 2200 Å.

III. APPLICATIONS

Numerous applications of spectroscopy to lipid problems have been made and the number is increasing rapidly. Citations here will be limited to a few examples from each category of use with fatty acids, excluding the many applications to the fat-soluble vitamins, hormones, sterols, pigments, etc., in which fields spectroscopy has been an invaluable tool of identification.

A. Analysis

In 1927 Manecke and Volbert first studied the absorption spectra of eleostearic acids, but not until 1934 did Dingwall and Thomson (12) show that the spectra of the acids were so different that the percentage composition of a mixture of the two could be determined spectroscopically.

For some years this laboratory has used spectroscopy for quantitative measurement of different conjugated systems (23, 27), as well as for the proof of the presence of such structures in mixtures. Spectroscopic measurements are unaffected by hydroxyl groups and others which interfere with the maleic anhydride diene number. Since they further distinguish between conjugated dienes, trienes, and tetraenes, spectroscopy is to be preferred to chemical methods of dienometry. Figure 15 compares the chemical and spectroscopic measurement of dienes in some commercial preparations and in two dienoic acids.

We have found that Moore's reaction (see below) can be made reproducible and hence an empirical quantitative measure of linoleic and linolenic acids in oils. In this reaction bonds are shifted to the conjugated position, giving an absorptive diene and a triene from linoleic and linolenic acids,

respectively. Unpublished results from this laboratory show the following content of linoleic acid in commercial oils: cottonseed, 57.5; corn, 65.1; almond, 21.5; peanut, 32.9; sunflower, 56.8; castor, 8.1; and olive, 17.2. The method will soon be checked carefully against standard chemical analyses. It is especially useful in showing the presence of a small amount of linolenic acid, which produces a new band at 2700 Å.

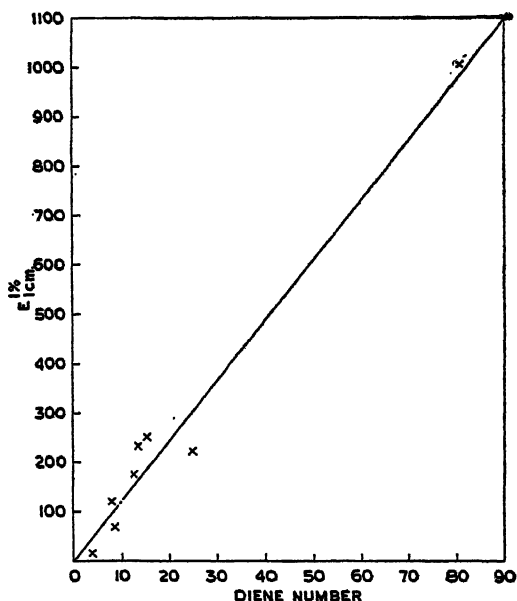


FIG. 15. $E_{1\text{cm}}^{1\%}$ at 2325 Å. plotted against diene number. The upper points are from some preparations of fairly pure 10,12-linoleic acids. The lower points are from commercial conjugated preparations.

B. Structure

In addition to the evidence as to conjugation, spectroscopy has been widely used to distinguish the *cis*- and *trans*-forms of ethylenic compounds. This change brings about a shift in the position of the absorption bands, but there is no agreement as to which form absorbs the longer wave lengths. Carr and Stüchlen (7) show that in the *cis*-forms of 2-butene and 2-pentene the first absorption band is shifted about 50 Å. toward the red and is very intense. This agrees with the finding of Hulst that the first band for oleic acid (*cis*) appears at longer wave lengths than for elaidic acid (*trans*). From this it would be assumed that α -eleostearic acid is the *cis*-form. Hartley (17) recently used a colorimetric measurement of the degree of conversion of *trans*-azobenzene to *cis*-azobenzene, made possible by the change in visible absorption.

Morton *et al.* (32) have shown the usefulness of the spectrographic method in determining the keto-enol isomerization of oxy acids.

C. Chemical reactions and changes during processing

In 1931 Gillam *et al.* (15) showed the presence in butter of spectroscopically active acids with an absorption maximum at 2300 Å. Booth *et al.*

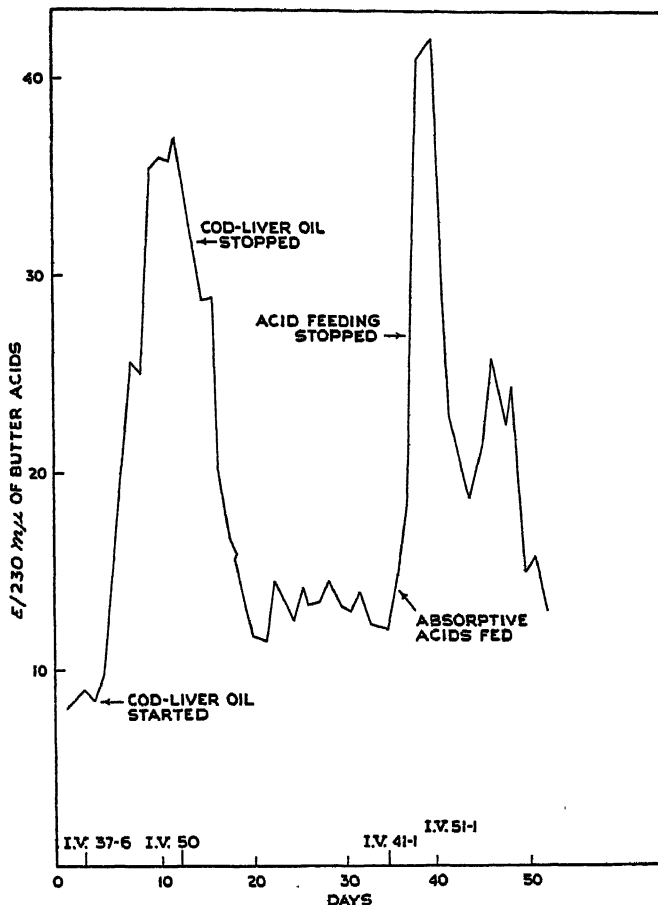


FIG. 16. Effect of feeding cod-liver oil and spectroscopically active acids on butter acids (from Dann *et al.*).

(4) demonstrated the seasonal variation of these acids, with absorption values ($E_{1\%}^{1\text{cm.}}$) running from about 25 in the summer to about 10 in the winter. The same authors (11) also demonstrated the effect of feeding cod-liver oil on butter fatty acids, shown in figure 16.

During the course of these investigations the above workers found that

prolonged saponification increased the absorption of the fatty acids. This led to an extensive investigation by Moore (29), who showed that the effect increased with unsaturation. Some of his data are given in table 1. He further showed that from linseed oil new acids were produced which resembled eleostearic acid. Edisbury *et al.* (13) applied the saponification technique to fatty acids of cod-liver oil and concluded that cyclization

TABLE 1
Effect of unsaturation on absorption

OIL	IODINE VALUE	$E_{1\text{ cm.}}^{1\%}$ AT 2325 Å. AFTER 24 HR. SAPONIFICATION
Coconut.....	8-9.5	2
Olive.....	79-88	12
Peanut.....	83-100	51
Cottonseed.....	108-110	120
Soybean.....	122	120
Linseed	171-201	170

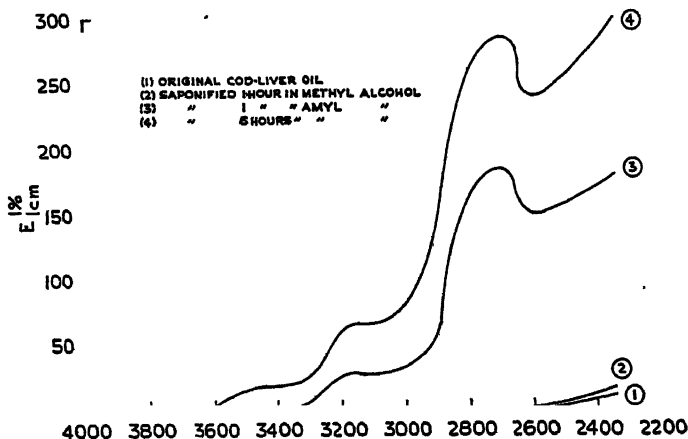


FIG. 17. Absorption spectra of cod-liver oil acids after prolonged saponification (drawn from Edisbury *et al.*).

of the natural C_{20} and C_{22} acids is the most plausible explanation of the changes in absorption spectra. The reaction takes place quickly at higher temperatures, as shown in figure 17.

Vacuum distillation of the methyl esters of highly unsaturated fatty acids brings about changes in the absorption spectra. Gillam *et al.* (15) found a decrease in absorption during distillation, presumably due to decomposition of the active groups. On the other hand, Norris *et al.* (33) have shown that there is some increase in absorption of the highly

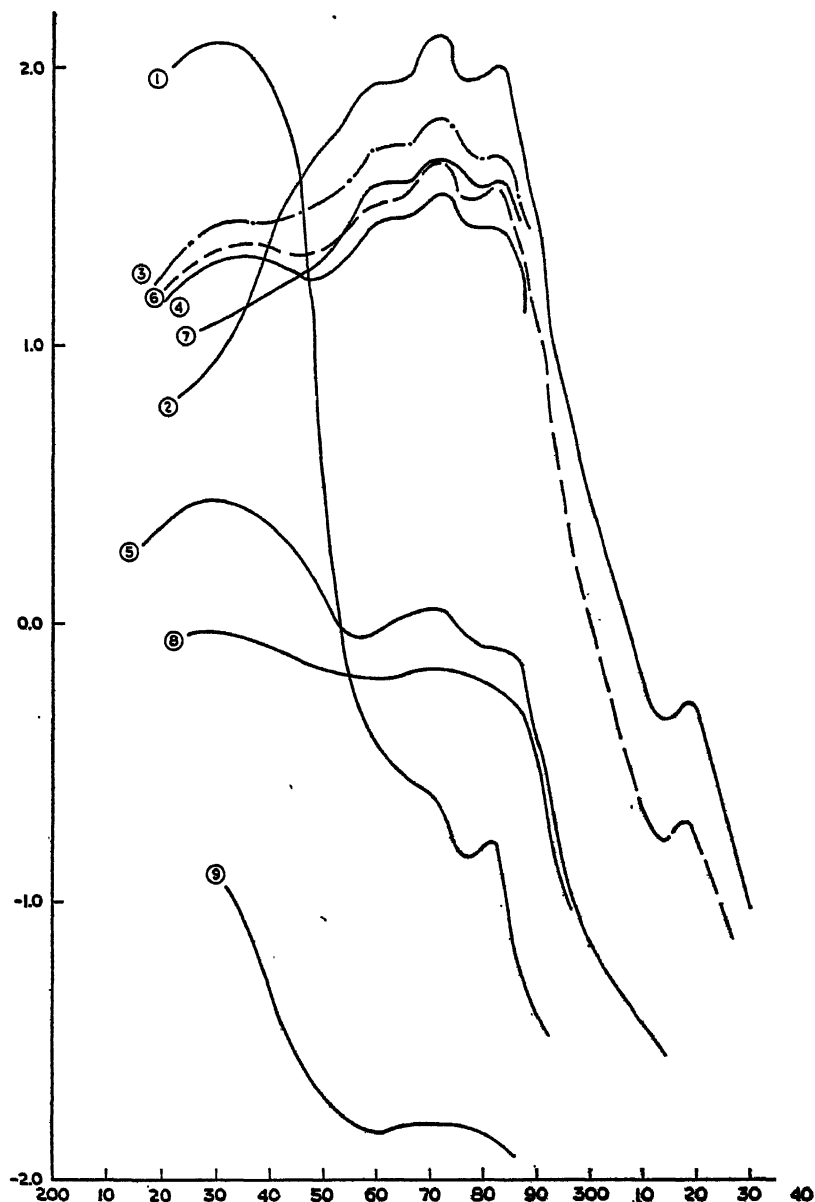


FIG. 18. Changes in absorption spectra of tung oil during hydrogenation (Hulst). Curve 1, 9,11-linolic acid; curve 2, China wood oil; curve 3, hydrogenation product A₁ (Normann); curve 4, hydrogenation product B₂ (Normann); curve 5, hydrogenation product C (Normann); curve 6, hydrogenation product b₂ (Wilbuschewitch); curve 7, hydrogenation product IX (high pressure); curve 8, hydrogenation product — (high pressure); curve 9, glycerols of palm oil.

unsaturated fatty acids of linseed and cod-liver oils due to the heating required for distillation. Bradley and Richardson (5) have also used the spectrographic method of following changes in drying oils during heating.

In 1935 Hulst (21) used absorption spectra for the detection of impurities in fatty acids and for following the changes in tung oil during hydrogenation.

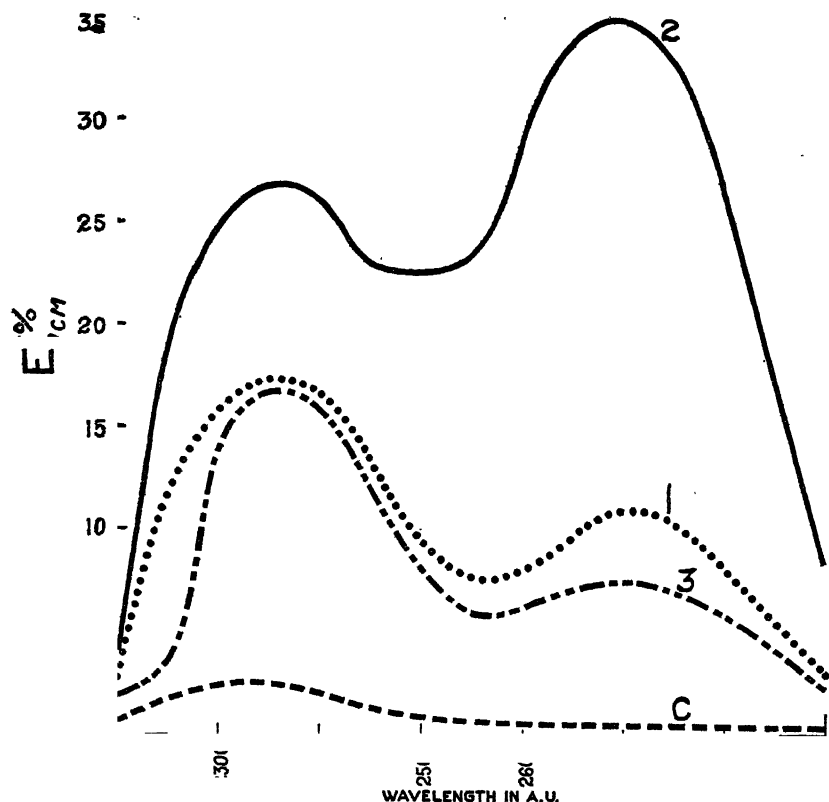


FIG. 19. Absorption curves of lipids extracted from adipose tissue of three experimental rats and a control. Curves 1, 2, and 3 represent rats killed 24, 48, and 70 hr., respectively, after consumption of the first tung oil. Curve C is the control on stock diet, but with no tung oil.

tion. Partial hydrogenation eliminates the absorption band at 2700 \AA. , with the production of a new band at 2300 \AA. (figure 18). Moore (31) has recently repeated the work for comparison with changes which take place when tung oil is fed to rats.

D. Biological tracers

The work of Moore and coworkers (11) showed that spectroscopically active fatty acids appear in butter when fed to cows. Following this

example Miller and Burr (28) fed tung oil to rats, which were killed and analyzed at daily intervals thereafter. The rate at which this material was deposited in the different tissues is indicated by the absorption spectra shown in figure 19. It seems that one of the first metabolic activities is the destruction of one double bond of the eleostearic acids (possibly hydrogenation), resulting in a dienoic acid with absorption maximum at 2300 Å. Moore (30) has recently observed the same phenomenon in the fat of hens' body tissues and eggs. Such changed fat does not appear in the milk of the cow after the feeding of tung oil.

Because of poor edibility and the above spectral changes, tung oil is not an ideal tracer. However, corn oil fatty acids which have been changed by our modification of Moore's reaction do serve well for such work. In this laboratory several papers (1, 2, 26) have been published which show the rates of transfer and phosphorylation of such fatty acids in normal and abnormal rats.

IV. CONCLUSION

With the improvement of apparatus for rapid quantitative measurements of absorption spectra in the far ultraviolet, many problems of lipid chemistry and physiology can be attacked in a more satisfactory manner than is possible without this tool.

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SYMPOSIUM ON REACTIONS AND EQUILIBRIA IN CHEMICAL SYSTEMS UNDER HIGH PRESSURE¹

INTRODUCTION TO THE SYMPOSIUM

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It is of interest to note that the American Chemical Society has reached its one-hundredth meeting before it has held a session devoted solely to that phase of physical chemistry which is concerned with the effects of high pressure on chemical reactions. From a detached thermodynamic point of view it may seem surprising that studies of the effects of temperature and of composition variations should so far have outrun studies of the effects of pressure variations because the three variables,—pressure, temperature, and composition,—are of coördinate thermodynamic importance in determining the state of a chemical system. From the practical point of view, however, we know that chemistry does not advance systematically but that its progress follows lines along which it is pushed by economic necessity, by externally stimulated curiosity, or by a burning zeal to overcome an opponent in a polemic. Natural fluctuations of temperature produce more profound effects on chemical systems than natural fluctuations of pressure, and we know that the large changes in reacting systems that may be wrought by an inexpensive Bunsen burner can only be matched by means of a very expensive piece of pressure apparatus.

While it would be interesting from an historical and philosophical point of view to follow this theme farther, our business in this Symposium is to take cognizance of the fact that interest in the behavior of chemical systems under pressure changes has increased in the last decade, and to consider some recent advances that have been made. By way of introduction to the papers that follow, I wish to call attention to six general fields in the study of material systems under high pressure that are of interest to chemists.

¹ This Symposium was held under the auspices of the Division of Physical and Inorganic Chemistry at the One-hundredth Meeting of the American Chemical Society, Detroit, Michigan, September 9–13, 1940.

GENERAL FIELDS IN THE STUDY OF MATERIAL SYSTEMS UNDER HIGH PRESSURE

1. The displacement of equilibria in homogeneous systems by rise of pressure

The displacement of equilibria in homogeneous systems by rise of pressure may result in larger yields of desirable products than can be obtained otherwise, if the reactions are sufficiently slow or the products readily removable. The use of this effect in gas reactions, especially the Haber process, is well known. Little work of this sort has been done with condensed systems which form the main subjects for very high pressure work. We may, however, mention the displacement of the tautomeric equilibrium of γ,δ -isheptenoic acid to the lactone form (16) and the effect of pressure on ionic equilibria, especially acid-base equilibria in sea water (13). The paper of B. B. Owen and S. R. Brinkley in this Symposium contributes to this aspect of pressure chemistry. Although the thermodynamic principles involved in this part of the subject have been well known for a long time, there is still need for development in their application to practical systems. The papers by L. J. Gillespie and by Manson Benedict and his coworkers deal with this question.

2. The displacement of equilibria in heterogeneous systems

The displacement of equilibria in heterogeneous systems, of which the change of solubility under pressure is a simple example, has been studied to a considerable extent, although it offers possibilities which have not yet been adequately exploited. The use of pressure changes in effecting separations by crystallization, particularly when the interactions of the solvent and the solutes to be separated are markedly different, is an example of this. For instance, the solubility of anhydrous sodium bromide in water increases significantly with a rise of pressure of 1000 bars, whereas the solubility of cesium bromide decreases markedly under the same conditions (17). The principles correlating the effect of pressure on solid-liquid equilibrium in condensed systems with other thermodynamic properties have been developed and applied to specific cases by E. Cohen and his school (14) at moderate pressures, and by L. H. Adams (1, 2) at high pressures.

The use of high pressure to confine volatile components in heterogeneous systems has led to results of great technical, geological, and chemical importance. The papers by H. H. Storch, R. Wiebe, and W. B. Kay deal with interesting technical and physicochemical aspects of this topic. Of special geological interest are the studies of the solubility of water in rocks and minerals at high pressures and temperatures made by R. W. Goranson (19), who has demonstrated that a rock like granite or a mineral such as albite can readily dissolve 10 per cent of its weight of water, and that the melting point of the rock or mineral is lowered several hundred degrees by

the dissolved water. L. H. Adams' paper will give a survey of high-temperature and high-pressure investigations of heterogeneous systems.

3. Acceleration or retardation of chemical reactions by pressure

During the last decade quite a number of examples of the effect of pressure on reaction velocities in condensed systems have been studied, especially by workers at the Imperial Chemical Industries Laboratory (16, 27, 33) and at Imperial College, London (26), and I regret that Professor Linstead could not be present to discuss the results of some of these investigations. In condensed systems pressure changes may produce large changes in reaction velocity, including the special case of reactions which do not go at atmospheric pressure, proceeding at finite rates under high pressures. For example, the workers at the Imperial Chemical Industries Laboratory found that the velocity constant of the addition of dimethylaniline to isopropyl iodide is increased some 500-fold by a rise of pressure of 12,500 atmospheres. This result is typical, if somewhat larger than the average, of the effect of pressure on "slow" reactions. They found that the effect of pressure on the rates of "normal" reactions, i.e., those in which the steric factor is close to unity, is much smaller and almost linear, while unimolecular decompositions were somewhat retarded by increase of pressure. The pioneering work of Bridgman and Conant on polymerization under pressure should also be mentioned. The paper by Eyring and Stearn discusses the effect of pressure on reaction velocities from a theoretical standpoint, and the paper by R. B. Dow, "The denaturation of proteins by high pressure," presents new work in a closely related field.

4. New phenomena

By extending greatly our range of observation, advances in high-pressure technique open up the possibilities of encountering new phenomena. New phases of solids existing only at high pressures, such as the five forms of ice, are now familiar from the work of Tammann (31, 32) and of Bridgman (5, 8), and, indeed, the latter's recent experiments (9, 10) up to 50,000 kg. per cm.² show that most solids have at least one polymorphic inversion in this range. Bridgman's experiments on the effect of shearing stress combined with high hydrostatic pressure (6, 7), by which he showed among other things that oxides such as lead dioxide or silver oxide decompose explosively under these conditions, have opened up an extremely important field in the chemistry of solids. Under this heading we may also class the work of Poulter on the penetration of liquids into glass under high pressures (29), and the recent work of Gibson and Loeffler on the effect of hydrostatic

pressure on the absorption of light by mixtures of certain organic compounds (18).

5. Knowledge of the behavior of naturally occurring substances under extreme conditions

Even at moderate depths within the earth, high pressures and elevated temperatures prevail, and in the depths of the ocean, pressures of 1000 atmospheres are reached. It is interesting to note that curiosity about geological and oceanographic phenomena, especially about the processes that have given the substance of the earth its present form, has provided the chief stimuli for piezochemical research. The best equation, in my opinion, that we have for representing the volume of a liquid as a function of the pressure was proposed by Tait in 1881, during his work on the observations of the Challenger Expedition. The work of Brander (4) on the effect of pressure on the conductivity of solutions and on acid-base equilibrium was also done under oceanographic auspices, and several articles of direct physicochemical interest are to be found in rather inaccessible journals devoted to marine studies (12). Under this general heading Dr. Berl's paper on "The rôle of carbohydrates in the formation of bituminous coals, asphalts, oils, and natural gas" is classified.

6. New methods of attacking old problems

Theories of matter in the gaseous state are largely based on pressure-volume-temperature observations, and studies of the effect of temperature and composition changes on condensed systems have provided much of the material employed in the theories of physical chemistry, especially those dealing with liquids and solutions. In principle, however, the results of temperature changes at constant pressure are less easy to interpret than the results of temperature changes at constant volume or volume changes at constant temperature, and, indeed, recent systematic investigations of the behavior of liquids and solutions under simultaneous changes of pressure and temperature have thrown valuable light on some outstanding problems in the physical chemistry of aqueous solutions. This aspect of pressure chemistry is still in its infancy.

INTERRELATIONS

In order to illustrate the place of the various types of high-pressure investigations in the general scheme of physical chemistry, and to answer the still frequent question "What do P - V - T measurements have to do with chemistry?", a chart showing the interrelations among the various activities has been prepared (see chart I).

APPARATUS

The development of the experimental technique of making accurate observations under high pressures is an important branch of this subject, and all investigators in this field acknowledge a deep indebtedness to Professor Bridgman, who has been in the forefront of all these developments for more than thirty years, and whose early experiments really made

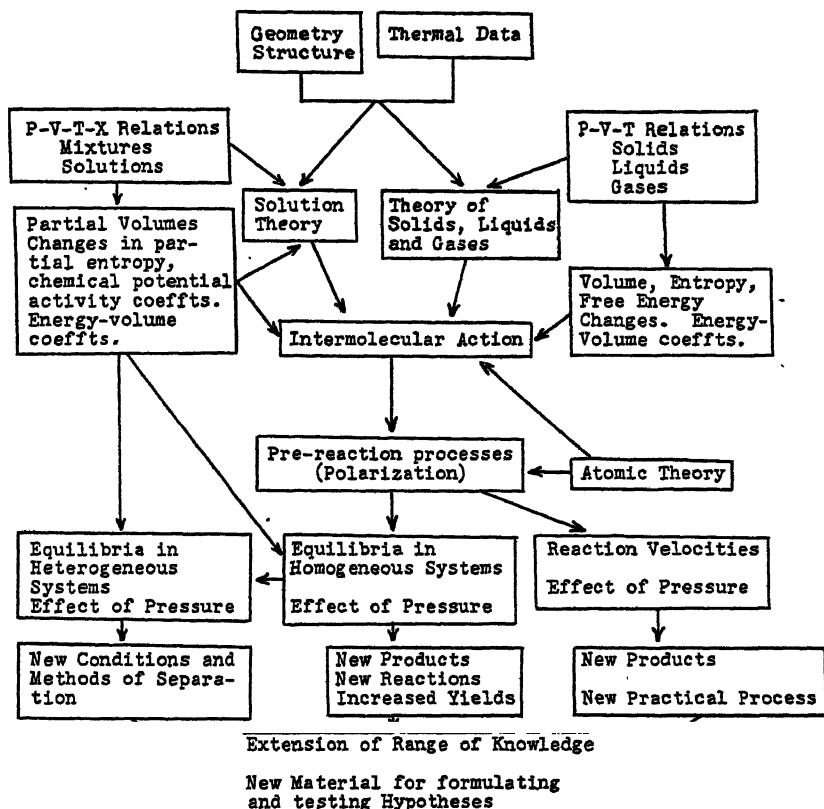


CHART 1

accurate high-pressure work possible. He has recently (11) extended these measurements up to 50,000 kg. per cm.² It is impossible to list all contributions to these experimental methods (25), but mention may be made of the apparatus for observing systems under pressures up to 4000 atmospheres at 1500°C., developed by Adams (30), and the simple but effective means of installing transparent windows in pressure bombs devised by Poulter (28).

Recently Griggs (24), working in Bridgman's laboratory, and Goranson (22), at the Geophysical Laboratory, established on experimental and theoretical grounds the principle that the strengths of materials increase rapidly when the hydrostatic pressure around them increases. This has led to the construction by Bridgman (12) and by Goranson and Johnson (23) of apparatus in which pressures exceeding 200,000 atmospheres have been generated. Dr. Goranson develops this subject with special reference to the design of high-pressure apparatus in his paper before the Symposium.

For a variety of reasons the following papers delivered at the Symposium are not published here (the figures in parentheses give references to articles in which the authors have discussed closely related subjects): "The denaturation of proteins by high pressure," by R. B. Dow (15); "The formation of natural gas, asphalts, oils, and coals in nature and in the laboratory and the importance of high pressure," by E. Berl; "Non-uniform pressure phenomena and their relation to the design of high-pressure apparatus," by R. W. Goranson (20, 21); "An empirical equation for thermodynamic properties of mixtures of light hydrocarbons under pressure," by Manson Benedict, George B. Webb, and Louis C. Rubin (3).

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EQUILIBRIUM IN HETEROGENEOUS SYSTEMS AT HIGH TEMPERATURES AND PRESSURES¹

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Following a summary of the principles involved in chemical equilibria, there is given a brief discussion of apparatus and experimental procedure for investigations in this field. Special attention is paid to systems that include a solid phase. The effects of pressure and of temperature are considered separately and in combination. A résumé is presented of certain experimental results obtained at pressures extending to several thousand atmospheres and at temperatures exceeding 1000°C. Reference is made to the application of these results to manufacturing processes and also to volcanology and other branches of geophysics.

I. INTRODUCTION

The present objective is to review briefly the principles by which chemical equilibrium is determined, and to note some representative results and methods in the investigation of equilibrium in systems of variable composition, especially at elevated temperatures and pressures. The range of these variables for which precise measurement is feasible has been greatly extended in recent years; there now exists a vast array of significant experimental results at pressures of several thousands of atmospheres and at temperatures up to 1000°C. or more. Such results, on the one hand, are of vital importance to various manufacturing processes, and, on the other hand, give promise of solving certain fundamental problems in geology and geophysics.

The present treatment of equilibrium is limited to heterogeneous systems and also to those involving one or more solid phases. This limitation is not imposed by any lack of importance of homogeneous systems, or of liquid-vapor or liquid-liquid systems. The whole field of equilibrium is too large a subject to be treated even sketchily in the space now available. It may be noted that liquid-vapor systems form a subject of special in-

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terest and importance, and one in which there have been so many new developments that detailed treatment elsewhere is desirable.

II. THE PRINCIPLES OF EQUILIBRIUM

The fundamental principles of equilibrium are best described in the language of thermodynamics. Chemical equilibrium has a basis similar to that for mechanical equilibrium, except that the variability in chemical composition requires the introduction of new parameters expressing the properties of the variable components. This means that a quantity, the chemical potential,—or its equivalent, the activity,—must be specified for each component and these chemical potentials combined with the other more familiar thermodynamic quantities, in order to fit the laws of physical chemistry into one simple framework.

In any system, and in any combination of phases, equilibrium is determined by the basic principle of the equality of temperature, of pressure, and of the chemical potentials. For any component the appropriate chemical potential must have identical values in adjacent phases; otherwise, an equilibrium state can not exist. In homogeneous systems this rule finds expression in the well-known Law of Mass Action, which in its exact form states that at constant temperature a certain product-quotient, involving the separate activities of the various components, remains constant. For heterogeneous systems an equilibrium constant can be specified, but it does not have a similar utility. In any system, however, thermodynamics makes its best contribution by supplying a quantitative basis for the study of equilibrium and a convenient measure of the extent to which reactions or processes go in one direction or the other. The quantity most often employed for this purpose is F , the free energy of a substance, or ΔF , the change in free energy that occurs during the transition from one state to another. Either quantity is determined directly from the chemical potentials. It happens also that ΔF is simply related to the equilibrium constant. Moreover, for any reaction or process it is a convenient criterion of equilibrium because its magnitude is a precise indication of the distance from the equilibrium state. Any reaction, chemical or physical, may be characterized by an equation which represents the transition from one state to another. If the substances on one side of the equation are in equilibrium with those on the other side of the equation, ΔF for the reaction is zero. A large negative value of ΔF means that the reaction as written tends to proceed nearly to completion and that therefore the substances on the left-hand side of the equation have a strong chemical affinity for each other. For example, at 1400°K. and 1 atmosphere the value of ΔF for the reaction by which 1 mole of carbon dioxide is formed from carbon monoxide and oxygen is -8040 calories. This signifies a strong affinity of

carbon monoxide for oxygen; even at 1400°K. carbon dioxide is dissociated to the extent of only 0.015 per cent. On the other hand, a large positive value of ΔF means that the reaction proceeds only slightly in the direction indicated. As an illustration, nitrogen and hydrogen, on combining to form ammonia at 1000°K. and 1 atmosphere, involve a change in F amounting to +14,800 calories per mole of ammonia. This gives quantitative expression to the reluctance, under these conditions, of nitrogen to combine with hydrogen; if the two gases are brought together in the stoichiometrical proportions, a maximum of 0.036 per cent will combine at the temperature and pressure under consideration to form ammonia.

The advantage of having a single quantity that conveniently and precisely describes equilibrium and the tendency to react, is obvious. This quantity is applicable to so-called physical reactions as well as to purely chemical reactions. If we are dealing with the solubility of a substance in a solution, and if we symbolize the act of dissolving by the equation $S \rightarrow L$, then at saturation ΔF is zero, and a large negative value of ΔF means undersaturation, i.e., a strong tendency to dissolve, whereas a positive value of ΔF is associated with a state of supersaturation. For example, the ΔF associated with the dissolving of 1 mole of sodium chloride in water at 25°C. is -2780 calories.

III. EFFECT OF TEMPERATURE

The change of equilibrium as the temperature varies is of great importance. The simple and well-known equation for expressing the effect of temperature on ΔF , and hence the effect of temperature on equilibrium, is

$$\frac{d\left(-\frac{\Delta F}{T}\right)}{dT} = \frac{\Delta H}{T^2}$$

in which H is the heat content or enthalpy, and ΔH is therefore the negative of the ordinary heat of reaction (ΔH being positive when heat is absorbed). Qualitatively, this equation means that if ΔH is positive, an increase of temperature makes ΔF more negative,—in other words, the reaction in question goes more nearly to completion. Possibly the most important of all the qualitative laws of physical chemistry is the generalization that endothermic reactions proceed best at high temperatures. On the other hand, if ΔH is highly negative, i.e., if the reaction heat as ordinarily expressed is large, then high temperature tends to inhibit the reaction. So much for the qualitative implications. Quantitatively the equation is even more important. It allows us to determine the behavior of reacting substances over a wide range of temperatures without making a large

number of measurements. Values at as few as two temperatures (although three or four are often preferable) may be used to establish the complete equilibrium-temperature curve. By a reversed application of the equation, heats of reaction and other thermal quantities may be determined by measurements of equilibrium at several temperatures.

IV. EFFECT OF PRESSURE

The equation $d\Delta F/dP = \Delta V$ characterizes the effect of pressure on ΔF and hence on the tendency of a given set of substances to react. In this equation ΔV is the aggregate change of volume that occurs when the reaction proceeds to completion in the direction indicated. Here, again, an interesting and important qualitative form may be given to the principle. This may be stated as follows: If ΔV is negative, an increase of pressure makes ΔF smaller, that is to say, makes the reaction as written proceed more nearly to completion. In other words, high pressure favors the existence of materials of small volume, i.e., high density. This is an interesting example of important relations that are in exact agreement with what one might conclude by intuition or common sense. It seems quite reasonable that pressure should tend, not only to produce a simple contraction of objects on which it acts, but also to squeeze them into other states of higher density. Like the corresponding equation for temperature, this relation, however, has an even more important quantitative use and is the basis, as will be shown later, of a powerful method for investigating equilibrium at extreme pressures. At the moment it is sufficient to note the interesting conclusion that, if there are two possible states of aggregation the second of which has the lower volume, then there is some pressure (possibly very high) at which the second state will predominate. We know, for example, without any measurement except of density, that diamond at a sufficiently high pressure is the stable form of carbon. It is certain also that garnets, which do not form at atmospheric pressure, must at a sufficiently high pressure be the stable form of the corresponding silicate mixtures.

V. DIRECT DETERMINATION OF EQUILIBRIUM AT HIGH TEMPERATURES

The essential requisites are a furnace for producing high temperatures and a means of measuring the temperatures with adequate precision. Owing to their convenience, electric furnaces of the wire-wound resistance type are commonly employed. For measurements that continue for a considerable period of time, an automatic temperature regulator is indispensable. A large number of types of such regulators have been devised, some depending upon the electromotive force of a thermocouple to actuate the regulating mechanism, and others using different principles. A re-

markably successful regulator used for many years at the Geophysical Laboratory works on the principle of the Wheatstone bridge, the furnace winding being one arm of the bridge and variations in temperature causing an unbalanced E.M.F. which by an appropriate mechanism regulates the current through the furnace so as to maintain the temperature at a constant value.

Without going into detail concerning the various methods of measuring high temperatures, it may be noted that for temperatures beyond the range of the mercury thermometer three principal types of instruments are used. These are the thermocouple, the resistance thermometer, and the radiation pyrometer. The platinum-rhodium thermocouple finds extensive use because, first, it is capable of high precision at temperatures up to 1700°C.—which covers the range of the greater part of the experimental studies in this field—and also because the thermocouple measures temperature practically at a point and is thus adapted for working with very small samples. A combination of platinum resistance furnace, automatic temperature regulator, thermocouple, and a potentiometer to read the electromotive force is considered by many to be the most generally useful equipment for the determination of equilibria at high temperatures.

Brief reference should be made to the various methods for determining equilibrium. Perhaps the most familiar one is the heating-curve method. In order to measure the melting point of a material, the time-temperature curve is taken while the material is raised up to and above the temperature at which it becomes liquid. In the ideal case the temperature as measured at a point within the material remains constant for a considerable period of time when the temperature has reached the point at which liquid and solid are in equilibrium. Many materials, notably silicates and other substances that form a viscous liquid, do not yield heating curves in which there is any region of constant temperature; but for such materials the melting point is nevertheless indicated, more or less clearly, by a change in direction of the curve.

A less familiar procedure is the so-called quenching method (6). This is applicable to material in which equilibrium is attained slowly and is peculiarly advantageous for such substances, because they do not yield heating curves with sharp indications of melting points or other inversions. The method has been applied with special success to the study of silicates. A small charge wrapped in platinum foil is suspended in an electric furnace and held at a constant temperature for a time necessary for equilibrium to be obtained. The charge is then dropped from the furnace into water or mercury, and the equilibrium is thus "frozen." Subsequent examination of the charge shows whether the material at the particular temperature was above or below the equilibrium point. Further trials allow an ap-

proximation to this temperature within successively narrowed limits. Although this method might at first thought appear somewhat crude when compared to the heating-curve method, it has been applied with great success to the investigation of difficult silicate systems.

VI. DIRECT DETERMINATION OF EQUILIBRIUM AT HIGH PRESSURES

Such measurements require a means for producing, maintaining, and measuring pressure. In essence the apparatus for producing pressure is a pump. This may take the form of an ordinary pump with valves and auxiliary equipment for actuating the pistons, or in the case of very high pressures may be a simple bomb and piston capable of utilizing only a single stroke of the piston. The production of leak-proof joints and packings is a complicated and difficult technique, but it is now a relatively simple matter to produce and control satisfactorily pressures up to 20,000 atmospheres or more. Pressures of twice this amount are obtained readily by supporting the outside of the bomb by means of a tapered ring (4), but much less accuracy and flexibility of performance are possible at these high pressures. Recently a greatly increased range of pressures has become available by utilizing the increase of strength caused by hydrostatic pressure. Preliminary experiments have indicated that pressures in excess of 200,000 atmospheres are attainable.

In any experimentation at extreme pressures an outstanding difficulty is the obtaining of a suitable medium for transmitting the pressure. Even at 20,000 atmospheres there are very few liquids that at room temperature do not become frozen, either in the sense of changing to a crystalline solid, or of becoming highly viscous or even glassy. Among ordinary liquids pentane seems to be the only suitable one for pressures of this magnitude. At somewhat lower pressures other hydrocarbon liquids may be employed, but at pressures much higher than 20,000 atmospheres there is no entirely satisfactory medium. A gas such as nitrogen and argon may be used up to the pressure (probably very high) at which a crystalline solid is produced, but there are practical difficulties in handling and storing the gases. For the present at least it appears that whenever we deal with pressures that exceed a few tens of thousands of atmospheres, we must be reconciled to the imperfect transmission of pressures associated with flow in so-called solid materials. This circumstance imposes an important limitation on the accuracy of measurements at extreme pressures.

There are two principal types of method that are suitable for measuring pressures that exceed a few thousand atmospheres. One method involves the force exerted on a piston, which may be either the piston that produces the pressure or a separate piston. Friction, especially when a packing is used, introduces a considerable uncertainty, although this can be reduced

by averaging the readings taken with increasing and decreasing pressure. The second general method, which is capable of high precision, makes use of the change in resistance of a metal exposed to hydrostatic pressure. This change is large enough to permit the determination of pressure to greater accuracy than that with which the pressure scale is now known. In manganin, which is the material commonly used, the relation between pressure and change in resistance is nearly linear up to several thousand atmospheres, if the gauge is properly constructed (3). At 25,000 atmospheres a departure from linearity of 1 or 2 per cent has been observed (5). It should be noted that at these higher pressures, the installation and maintenance of the resistance-pressure gauge becomes increasingly difficult, on account of the necessity of introducing the electric leads through insulated and pressure-tight packings. The estimation of pressures much above 25,000 atmospheres is usually carried out through a calculation involving the force exerted on a piston and its area.

There are several methods for determining the behavior of materials under pressure and thus obtaining information as to equilibria. One of the most important is the volume-change method, which is commonly used to detect a transition under pressure from one state to another. The procedure is merely to make simultaneous pairs of readings of the pressure and of the piston position; measurements can be made with high precision. The volume-change method, therefore, gives a delicate indication of transformations, such as the melting of a solid, the dissolving of one phase in another, and other changes such as polymorphic transformation. This method is analogous to the heating-curve method for investigations at elevated temperatures. In using the heating-curve method we are in effect measuring temperature and entropy, whereas in the volume-change method for pressure experimentations we are utilizing the thermodynamically comparable pair of factors, pressure and volume.

VII. EQUILIBRIUM AT HIGH TEMPERATURES COMBINED WITH HIGH PRESSURES

When the difficulties incident to high-pressure experimentation are superimposed upon those incident to high temperatures, the necessary technique has appeared to be quite formidable. Much care in the design of the apparatus was necessary, and one must expect to spend a considerable amount of time in keeping the apparatus in running order. There are two principal varieties of apparatus for combined high temperature and high pressure. The first uses external heating, and the second is arranged for internal heating by means of an electric heating coil.

An example of a bomb with external heating is that used by Morey (9) in the investigation of the behavior of silicates in the presence of water

under pressure. The bomb is a simple cylinder closed at the bottom and provided with a packing nut at the top. A sample of material to be studied, contained in a gold or platinum crucible, is placed within the bomb, together with the appropriate amount of water, so that at the chosen temperature the desired pressure will be developed by the expansion of the water. This apparatus is especially well suited for experiments with materials which quench readily. In its simplest form no means are provided for measuring directly either the temperature or the pressure within the bomb. The pressure is calculated from the amount and temperature of the fluid used, and the temperature is measured by means of a thermocouple inserted in a small hole in the wall of the bomb. Pressure and temperature are limited by the characteristics of the metal used. In the newer types of stainless steels the practicable limit is of the order of a few hundred atmospheres pressure at a temperature of 600 to 800°C.

An example of interesting work carried out by the simple bomb with external heating is found in the ternary system water-potassium silicate-silica, investigated by Morey and Fenner (10). Equilibrium with various mixtures was determined by what has been called a hydrothermal quenching method. The essence of this method is to use a rate of cooling, neither too great nor too small, such that the cooled sample preserves the equilibrium conditions existing at the chosen temperature and pressure. It was found possible to delineate the stability fields in the ternary system and to determine the variation of vapor pressure with the change in ratio of silica to potash. With a more elaborate type of apparatus measurements have been obtained in the system soda-silica-water. Of especial interest are the results on the lowering of the melting point of $\text{Na}_2\text{O} \cdot \text{SiO}_2$ by the action of water at various pressures (11).

An example of high-pressure apparatus with internal heating is that used by Smyth and Adams (12). The novel features of this bomb were, first, the use of a hydraulic press to hold the lid on the bomb, and second, the cooling of the walls by circulating water through spiral grooves which were turned on the outside of the inner shell before the assembling of the inner and outer shells. Heating was attained by means of a small resistance furnace made by winding platinum or platinum-rhodium wire on a refractory tube and surrounding the tube by a suitable insulating material. For such apparatus the choice of this insulating material is very important. It must be a poor conductor of heat and at the same time not be so fluffy that it will be displaced or blown away by the violent currents due to addition or removal of the pressure fluid. Magnesium oxide, which is an excellent substance for ordinary electric furnaces, is not usable, because it is too light. Granular aluminum oxide (alundum) is a fair thermal insulator and fulfills the requirements of resisting satisfactorily the disturbances accompanying the increase or release of pressure.

The medium originally used to transmit pressure was carbon dioxide. More recently nitrogen has been substituted for the carbon dioxide and has been found to have the advantage of a comparatively small (effective) thermal conductivity. This is an important feature, because it is always difficult to attain high temperature in a region surrounded by a gas under pressure. At pressures of several thousand atmospheres all gases have a density comparable with ordinary liquids, and the problem of maintaining elevated temperatures in a gas at high pressure may be compared to using a resistance coil to obtain a red heat in the middle of a beaker of water. Both conduction and convection enter into the loss of heat, and in both these respects nitrogen is a great improvement over carbon dioxide.

Through the lower lid of the bomb are brought electrically insulated leads for supplying the furnace with current, and a number of leads are also supplied for connecting one or more thermocouples to the interior of the electric furnace. Nitrogen under pressure may be pumped into the bomb by means of a three-stage motor-driven compressor. The apparatus is suitable for experimentation at pressures up to 4000 atmospheres and at temperatures up to 1500°C.

An example of equilibrium measurements obtained with the externally heated bomb is given by the work on the system calcium oxide-carbon dioxide, for which Smyth and Adams (12) determined the melting relations and vapor pressures. At carbon dioxide pressures of 40 atmospheres or more, calcium carbonate will melt if the temperature is raised sufficiently. Under 40 atmospheres pressure melting begins at 1240°C. The melting point, however, is an incongruent one, the composition of the liquid not being that of pure calcium carbonate but containing an excess of calcium oxide. To attain a nearly congruent melting would require a very much higher pressure than 40 atmospheres, but the fusion temperature would probably not be much above 1340°C.

Transformation in the solid state may be investigated by the heating-curve method in an apparatus of this kind. Precise determinations on the effect of pressure on the α - β inversion in quartz have been made by Gibson (7). Another type of measurement made with a similar apparatus is illustrated by the work carried out by Goranson (8) on the solubility of water in granite magma. A bomb with the same general design was used, but the device for cooling the walls of the bomb consisted of a thin space between the inner and outer walls, rather than a spiral groove as in the original apparatus. Experiments were conducted by sealing small charges of granite with the requisite amount of water in small platinum tubes and exposing these for a sufficient length of time to the desired temperature and to a pressure sufficiently high to counteract the tendency of the water within the sealed tube to expand. The walls of the tube being thin (of the order of 0.1 mm.), there was no important difference of pressure between

the inside and the outside of the tube. This investigation extended to pressures of about 4000 atmospheres and temperatures of about 1000°C. The solubility of water first rises rapidly with pressure, and then more slowly. It is interesting to observe that at a moderate pressure it is possible to have water to the extent of nearly 10 per cent by weight dissolved in the silicate mixture having the chemical composition of a typical granite.

VIII. INDIRECT, OR THERMODYNAMIC, DETERMINATION OF EQUILIBRIUM

This method depends on the circumstance that equilibrium is determined by free energy and that the free energy in turn becomes known at all pressures if the volumes and entropies of the individual phases can be determined at the corresponding pressures. The thermodynamic method is especially advantageous when pressure and composition are the variables. For such systems the method, although an indirect one, is capable of extraordinary precision and, in general, represents perhaps the most satisfactory procedure for studying equilibria at very high pressures. In this instance the experimentation reduces itself mainly to the measurement of specific volumes or densities. The method presupposes a knowledge of the state of the system at one pressure, say atmospheric, and it involves merely a measurement of the compressibilities of all the separate phases that occur in the system.

A striking example of the application of the thermodynamic method to equilibrium studies is found in the phase diagram for the system sodium chloride–water at 25°C. This diagram was worked out (1) from measurements of the compressibilities of the solid phases involved (ice, sodium chloride, and sodium chloride dihydrate) and of a series of aqueous sodium chloride solutions of varying concentrations. Another system investigated by a similar technique is the system ammonium nitrate–water at 25°C. Here a striking decrease in solubility occurs (2), reducing the solubility of the salt from 67 per cent by weight at atmospheric pressure to 26 per cent at 12,100 bars, which is the pressure at which the solubility curve for ammonium nitrate intersects that for ice γ_1 .

In a strictly analogous manner, the solubility curves for cases in which temperature and concentration are the variables can be determined thermodynamically. Here entropy and temperature take the places of volume and pressure, and for the measurement of compressibilities we substitute the measurement of specific heats of the various phases, including a number of solutions of various concentrations. As before, we start with a known thermodynamic state at some arbitrary temperature and at the chosen constant pressure. A knowledge of the temperature at which each phase makes its appearance is also required. This information does not neces-

sarily come from direct observation, but, as is well known, can in some instances be determined by application of the Third Law of Thermodynamics through the utilization of thermal data. It should be noted that, although the bases for the indirect determination of equilibrium curves at either high temperatures or high pressures are essentially the same, in actual practice the indirect method seems to be of more value for high-pressure equilibria. One reason for this is that volume lends itself to precise measurement in stationary systems, whereas entropy and other thermal quantities are commonly measured in a moving system.

It is interesting to compare the type of diagram which represents the change of a system at varying pressure and constant temperature with the more familiar freezing point-solubility diagram, in which temperature and concentration are the variables and which refers to some constant pressure, usually atmospheric. Both kinds of diagrams contain curves which represent the equilibrium between solid and liquid and are called solubility curves, and in both types of diagrams the full area is divided into various fields representing the existence of different phases. The principal difference in appearance is that the pressure diagram appears to be upside down, the freezing-point curves *rising* until they meet in eutectics or other invariant points. This illustrates an interesting conclusion,—namely, that in general increasing pressure produces the same kind of effect as decreasing temperature. The volume of a substance, for instance, becomes greater at increased temperatures and decreased pressures. Solubility increases, as a rule, with increasing temperature, but in general is diminished by high pressure.

These newer methods furnish a striking example of the approach to heterogeneous equilibrium by the study of homogeneous equilibrium. By this powerful technique, measurements of volume and entropy at various pressures, temperatures, and concentrations form the experimental framework from which a complete knowledge of a system is derived by straightforward computation. Interrelations between the four quantities P , V , T , and X thus become of paramount importance. In the practical application of the thermodynamic method great difficulties appear to arise, principally because the quantities used in the study of solutions are the fictive or *partial* quantities. These, being determined in general from rates or slopes, entail a loss of accuracy in comparison with the primary measurements. The solution of the difficulty, of course, is high precision in the measurements, and fortunately this can readily be secured for measurements of volume even at very high pressures. It is significant that modern thermodynamics from the practical side, as applied to physical chemistry, consists mainly in the devices for the representation of data so as to utilize the basic thermodynamic relations.

IX. CONCLUDING REMARKS

From what has been said here concerning the determination of equilibrium at high temperatures and high pressures in systems containing a solid phase, it is evident that great experimental difficulties are involved and that these difficulties are gradually being nullified by new experimental and calculational methods in which volume plays an important rôle.

One of the interesting practical applications of studies of equilibria at high temperatures is in the field of refractories. The essential constituents of refractories overlap those which are of interest in petrology and include, besides silica, a number of oxides such as lime, magnesia, soda, potash, alumina, and the oxides of iron. Various combinations of these oxides have been studied, and numerous binary and ternary systems have been methodically investigated. Such measurements are immediately applicable, not only to problems of petrology, but also to the production of refractories. The important property of a refractory is, of course, its melting behavior, not only alone but in contact with other materials. It is the beginning of melting, rather than the temperature of complete melting, that is of most interest. This beginning of melting is described in terms of the eutectic temperatures of the system involved. Now that quantitative data are on hand for many of the essential mixtures, it is easy to understand a number of phenomena that formerly were quite puzzling. In materials used for spark plugs or for furnace linings and for other purposes, we can tell exactly the temperature at which softening begins and can follow the subsequent extent of the softening through study of the course of the melting curves. The practical value of such studies has been, first, the ability to make quantitative comparisons between refractories of various compositions, and second, the aid in making new and more valuable combinations.

A quite different application of indirect rather than immediate value has been to petrology and especially to volcanology. Volcanoes have attracted the attention of students of geophysics, because they give us direct evidence of a hot and active interior of the earth and because their behavior gives us a clue to the nature of the interior and of those processes by which we believe the earth itself was formed. The most striking feature of volcanic emanations, aside from the tremendous flows of molten magma, is the vast amount of volatile material discharged from volcanic vents. In these gaseous emanations, as also in those from fumaroles, water is the dominant constituent. We believe that water is an important and essential ingredient of the original or primeval magma whose consolidation has given rise to the visible crust of the earth. Since water is associated with silicate mixtures even at considerable depths, it is natural that an understanding of the primeval magma can be obtained only through a careful

study of silicate mixtures in which water is present as a major constituent. Such studies involve apparatus for combined high temperatures and pressures such as have been described above. Hardly more than a beginning has been made in this line of experimentation. Much more needs to be done, but already we know something of the behavior of aqueous silicate mixtures upon cooling, and we can understand something of the way in which the cooling can act to build up great pressures sufficient for volcanic outbursts and the way in which the varied assortment of minerals present in geologic formations can be found. It is evident that an understanding of the fundamental problems of geology, such as volcanism and related phenomena, depends upon further complete and systematic investigation of the equilibrium of silicate mixtures at temperatures and pressures comparable with those prevailing at depths of several hundred miles below the surface of the earth.

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CALCULATION OF THE EFFECT OF PRESSURE UPON IONIC EQUILIBRIA IN PURE WATER AND IN SALT SOLUTIONS¹

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Tables of standard partial molal and ionic volumes, and their pressure coefficients, are given for aqueous solutions at 25°C. These are used to estimate the effect of pressure upon the ionization constants of water and weak acids, and the solubility constants of several minerals. A pressure of 1000 bars causes a two- to eight-fold increase in these quantities at 25°C. The increase at other temperatures is briefly discussed, and rough values of ΔH° and ΔS° for the ionization of water are recorded as a function of pressure.

To give the results oceanographic interest, similar tables and calculations are presented for "salt water" (0.725 molal sodium chloride) of the same ionic strength as sea water. The calculations feature a shift of the standard states to "salt water," and the use of empirical methods of estimating standard volumes and their pressure coefficients in this medium. The effect of pressure upon ionic equilibria in "salt water" is less than in pure water.

The general thermodynamic relation which describes the effect of pressure upon the equilibrium constant is well known, but is rarely applied to condensed systems involving ions. This neglect of the important variable, pressure, is largely due to the scarcity of reliable values of the partial molal volumes and compressibilities of ionic solutes, especially in solutions containing several electrolytes. The present review is concerned with compiling preliminary tables of these quantities, and describing methods by which they may be evaluated from existing data. Their use will be illustrated by calculating the effect of pressure upon several ionic equilibria of interest to geologists and oceanographers.

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I. THE SOLVENT MEDIUM IS PURE WATER

The variation of the thermodynamic equilibrium constant with pressure is given by

$$RT \left(\frac{\partial \ln K}{\partial P} \right)_{T,m} = -\Delta \bar{V}^0 = - \left[\sum^p \bar{V}_2^0 - \sum^r \bar{V}_2^0 \right] \quad (1)$$

$\Delta \bar{V}^0$ is the algebraic difference between the partial molal volumes of the products and the reactants in their standard states. A component which takes part in the reaction as a pure phase is in its standard state by convention. The standard state of a solute is the hypothetical 1 molal solution in which its activity coefficient is unity. The partial molal volume of such a component is equal to its value at infinite dilution of all solute species. These conventions permit variations of pressure at constant composition, as required by equation 1.

For any solute, \bar{V}_2^0 can be obtained by extrapolating the apparent molal volume,

$$\phi_v = \frac{M_2}{d_0} - \frac{1000}{d_0} \left(\frac{d - d_0}{c} \right) \quad (2)$$

or other (12, 17) suitable function of the density, to infinite dilution. M_2 is the molecular weight of the solute, and c its concentration in moles per liter of solution. The densities of the solution and of the pure solvent are represented by d and d_0 , respectively. By definition

$$\phi_v^0 \equiv \bar{V}_2^0 \quad (2a)$$

For ionic solutes the extrapolation is based upon the observation (34, 36) that ϕ_v varies linearly with \sqrt{c} in dilute solutions, or

$$\phi_v = \phi_v^0 + S_v \sqrt{c} \quad (3)$$

For non-electrolytes (19, 35) and weak electrolytes (9, 21), ϕ_v varies linearly with c and the degree of dissociation, respectively. Table 1 contains values of $\bar{V}_2^0 (= \phi_v^0)$ for representative electrolytes at 25°C. and 1 atmosphere. The discordance between independently determined values for most of these salts is largely due to uncertainties in extrapolation, since an error of 0.001 per cent in density produces an error of 0.01/ c cc. in ϕ_v . Independent extrapolations (8, 37) of the same data (6) often disagree by ± 0.1 cc. The figures printed in bold face are the most self-consistent values we could obtain by imposing the condition of additivity of partial molal ionic volumes at infinite dilution, and are probably accurate to ± 0.2 cc., unless rounded off to the nearest cubic centimeter.

Before equation 1 can be used over any considerable range, $\Delta \bar{V}^0$ must be expressed as a function of P . If the partial molal compressibility (18),

TABLE 1

Partial molal volumes of electrolytes at infinite dilution in water at 25°C. and 1 atmosphere

ELECTROLYTE	S_0	\bar{V}_2^0	REFER- ENCE	ELECTROLYTE	S_0	\bar{V}_2^0	REFER- ENCE
HCl	0.95	18.07	(42)	KI	1.56	45.36	(37)
18.1		17.98	(5)	45.3		45.23	(8)
		18.20	(8)			45.28	(12)
LiCl	1.49	17.00	(37)			45.34	(33)
17.1		17.06	(8)	KOH	4.35	2.9	(18)
LiBr	1.16	24.08	(37)	3.4		4.06	(3)
24.0		24.07	(8)	KNO ₃	2.30	38.18	(15)
LiI	0.85	35.50	(37)	38.0		37.6	(30)
35.6		35.60	(8)			37.98	(33)
LiOH	3.00	-6.0	(18)	KClO ₃		46	(30)
-6.3				KBrO ₃		44	(30)
NaCl	2.15	16.40	(8)	KHCO ₃	2.6	32.7	*
16.6		16.28	(37)	K ₂ CrO ₄	11.7	37.1	(24)
		16.61	(31)	K ₂ SO ₄	12.07	32.28	(43)
		16.35	(12)	31.9		32.36	(27)
		16.67	(42)			32.30	(1)
		16.62	(23)			31.96	(24)
		16.44	(15a)	RbCl	2.22	31.87	(37)
NaBr	1.76	23.51	(37)	31.8		31.71	(8)
23.5		23.45	(8)	RbBr	2.04	38.71	(37)
		23.48	(9)	38.7		38.70	(8)
		23.45	(15a)	RbI	1.62	50.31	(37)
NaI	1.35	35.10	(37)	50.3		50.40	(8)
35.1		35.00	(8)	CsCl	2.17	39.15	(37)
		35.09	(15)	39.2		39.02	(8)
NaOH	4.18	-6.7	(18)	CsBr	1.90	46.19	(37)
-6.8		-5.94	(4)	46.1		46.20	(8)
NaO ₂ CCH ₃	1.9	40.1	*	CsI	1.58	57.74	(37)
40.0		39.72	(33)	57.7		57.90	(8)
NaHSO ₄	1.8	27	*			57.54	(26)
Na ₂ SO ₄	12.16	11.52	(9)	NH ₄ Cl	1.45	35.98	(30)
11.5		11.10	(12)	36.0		36.26	(33)
		11.39	(33)	NH ₄ NO ₃	0.97	47.56	(20)
Na ₂ CO ₃	11.30	-6.74	(9)	47.2		47.24	(9)
KF	3.35	6.60	(8)			47.49	(2)
KCl	2.33	26.52	(37)	AgNO ₃	2.61	28.01	(24)
26.8		26.36	(8)	28.3		28.76	(33)
		26.57	(27)	MgCl ₂	4.26	15.3	(38)
		26.87	(30)	CaCl ₂	6.00	18.25	(18)
		26.74	(43)	18.5		18.54	(33)
		26.65	(33)			18.6	(38)
		26.81	(9)	SrCl ₂	?	17.94	(31)
KBr	1.94	33.73	(37)	18.0		18.6	(38)
33.7		33.54	(8)	BaCl ₂	4.83	23.60	(18)
		33.97	(43)	23.9		24.1	(25)
		33.56	(22)	CdCl ₂	5.9	23.2	(33)
		33.88	(33)	LaCl ₃	11.87	16.0	(28)
		33.77	(29)	CH ₃ CO ₂ H		50.7	*
KMnO ₄		51.7	(26)				

* International Critical Tables.

$$\bar{K}_2^0 = -\left(\frac{\partial \bar{V}_2^0}{\partial P}\right)_{T,m} \quad (4)$$

of each component is known at the standard pressure, 1 atmosphere,³ it is satisfactory for many purposes to consider \bar{K}_2^0 constant over a moderate pressure range.⁴ In this case the partial molal volume at any pressure (P) is given by

$$\bar{V}_2^{(P)} = \bar{V}_2^0 - \bar{K}_2^0(P - 1) \quad (5)$$

and substitution in equation 1 leads to

$$RT \ln (K^{(P)}/K) = -\Delta \bar{V}^0(P - 1) + \frac{1}{2} \Delta \bar{K}^0(P - 1)^2 \quad (6)$$

upon integration.

A more complete expression, applicable at higher pressures, may be derived from the relation,

$$\bar{V}_2^{(P)} - V_2^{(P)} = \frac{-A}{B + P_e + P} \cdot \frac{dP_e}{dm} \quad (7)$$

obtained by Gibson (11). This relation is an extension of the Tait (39) equation, and is based upon Tammann's (40) hypothesis that, in the presence of an ionized solute, the solvent behaves as though it were subjected to a constant effective pressure, P_e , in addition to the external pressure, P . P_e is a property of the solution and a function of the concentration. The constants A and B are characteristic of the pure solvent.⁵ A , B , and P_e are independent of P . V_2 represents the molal volume of the pure solute in the liquid state.

At any given concentration and temperature, differentiation of equation 7 yields

$$\bar{K}_2^{(P)} - K_2^{(P)} = \frac{-A}{(B + P_e + P)^2} \cdot \frac{dP_e}{dm} \quad (8)$$

at the pressure P . Since K_2 , the molal compressibility of the pure liquid electrolyte, should not differ greatly from that of the solid, we shall assume that K_2 is negligible compared to \bar{K}_2 at all concentrations and pressures with which we are concerned. Therefore elimination of $A dP_e/dm$ between equation 8, and itself rewritten for $P = 1$, leads to

³ For condensed systems at ordinary atmospheric pressures it will be unnecessary to distinguish between 1 bar and 1 atmosphere.

⁴ It can be shown by equation 10 that an increase of 100 bars decreases \bar{K}_2^0 less than 7 per cent in aqueous solution at 25°C.

⁵ For aqueous solutions at 25°C., $B = 2996$ bars and $A = 434.3 \times 0.3150$ cc. per kilo of water (10); see reference 11.

$$\bar{K}_2^{(P)} = \bar{K}_2 \left[\frac{B + P_e + 1}{B + P_e + P} \right]^2 \quad (9)$$

at any concentration, or

$$\bar{K}_2^{0(P)} = \bar{K}_2^0 \left[\frac{B + 1}{B + P} \right]^2 \quad (10)$$

at infinite dilution. This equation relates $\bar{K}_2^{0(P)}$ to \bar{K}_2^0 in a very simple manner, and its integration results in the expression

TABLE 2

Partial molal compressibilities of electrolytes at infinite dilution in water at 25°C. and 1 atmosphere

ELECTROLYTE	$10^4 \bar{K}_2^0$	REFER- ENCE	ELECTROLYTE	$10^4 \bar{K}_2^0$	REFER- ENCE
HCl.....	-8	(20)	Na ₂ CO ₃	-171	(13)
LiCl.....	-42	(20)	K ₂ CrO ₄	-139	(13)
NaCl.....	-52	(20)	Li ₂ SO ₄	-146	(14)
KCl.....	-45	(20)	Na ₂ SO ₄	-154	(14)
LiOH.....	-78	(20)	K ₂ SO ₄	-139	(14)
NaOH.....	-89	(20)	Cs ₂ SO ₄	-119	(14)
KOH.....	-81	(32)	(NH ₄) ₂ SO ₄	-91	(14)
KO ₂ CCH ₃	-47	(32)	BaCl ₂	-115	(13)
LiCl.....	-41	(13)	CaCl ₂	-87†	(18)
NaCl.....	-48	(13)	MgSO ₄	-153	(14)
KCl.....	-44	(13)	CdSO ₄	-127	(14)
NaBr.....	-42	(13)	CuSO ₄	-132	(14)
KBr.....	-34	(13)	ZnSO ₄	-140	(14)
CsBr.....	-25	(13)	BeSO ₄	-93	(14)
LiI.....	-17	(13)	CeCl ₃	-176	(13)
KI.....	-18	(13)			
KONS.....	-22	(13)			
KNO ₃	-30	(13)	HO ₂ CCH ₃	+7	(13)
KHCO ₃	-35	*	H ₂ O.....	+8.22	(13)

* *International Critical Tables.*

† At 30°C. and 100 bars.

$$\bar{V}_2^{0(P)} = \bar{V}_2^0 - \bar{K}_2^0 \left[\frac{(B + 1)(P - 1)}{B + P} \right] \quad (11)$$

for $\bar{V}_2^{0(P)}$ as a function of the pressure. By combining this expression with equation 1 and integrating, we obtain the desired relation,

$$RT \ln (\bar{K}^{(P)}/\bar{K}) = -\Delta \bar{V}^0(P - 1)$$

$$+ \Delta \bar{K}^0 \left[(B + 1)(P - 1) - (B + 1)^2 \ln \left(\frac{B + P}{B + 1} \right) \right] \quad (12)$$

by which $K^{(P)}$ is calculable at pressures as high as 1000 atmospheres, or more.

The individual values of \bar{K}_2^0 can be obtained in a manner (18) analogous to that used in determining \bar{V}_2^0 , but it is more convenient to employ the expression

$$\bar{K}_2^0 = - \left[\frac{\bar{V}_2^0(P_2) - \bar{V}_2^0(P_1)}{P_2 - P_1} \right] \frac{(B + P_2)(B + P_1)}{(B + 1)^2} \quad (13)$$

derived from equation 10 by integration over any pressure interval, P_1 to P_2 . Since P_1 may be assigned the value unity, equation 13 permits the

TABLE 3

Partial molal ionic volumes and compressibilities at infinite dilution in water at 25°C. and 1 atmosphere
(Relative to H⁺)

CATION	\bar{V}_i^0	$10\bar{K}_i^0$	ANION	\bar{V}_i^0	$10\bar{K}_i^0$
H ⁺	0	0	OH ⁻	-5.3	-44
Li ⁺	-1.0	-34	F ⁻	-2.1	
Na ⁺	-1.5	-42	Cl ⁻	+18.1	-8
K ⁺	+8.7	-37	Br ⁻	+25.0	+2
Rb ⁺	+13.7		I ⁻	+36.6	+18
Cs ⁺	+21.1	-27	CH ₃ CO ₂ ⁻	+41.5	-10
NH ₄ ⁺	+17.9	-11	NO ₃ ⁻	+29.3	+7
Ag ⁺	-1.0		CNS ⁻		+15
Mg ⁺⁺	-20.9	-83	HCO ₃ ⁻	+24	+2
Ca ⁺⁺	-17.7	-71	MnO ₄ ⁻	+43	
Sr ⁺⁺	-18.2		ClO ₄ ⁻	+46	
Ba ⁺⁺	-12.3	-99	BrO ₃ ⁻	+44	
Be ⁺⁺		-23	CrO ₄ ⁻	+19.7	
Cd ⁺⁺	-13	-57	CO ₃ ⁻	-3.7	-85
Cu ⁺⁺		-62	SO ₄ ⁻	+14.5	-70
Zn ⁺⁺		-70			
La ⁺⁺⁺	-38.3				
Ce ⁺⁺⁺		-152			

calculation of \bar{K}_2^0 from density measurements at a single high pressure, P_2 . Table 2 contains values of \bar{K}_2^0 at 25°C. and 1 atmosphere. Those values which have not been previously reported were calculated by equation 13.

Values of the partial molal ionic volumes, \bar{V}_i^0 , and partial molal ionic compressibilities, \bar{K}_i^0 , are given in table 3. These properties of the individual ions are relative, and are obtained by setting the partial molal volume and compressibility of the hydrogen ion equal to zero. This convention is analogous to that used in tabulating ionic entropies, and is very convenient for numerical calculations. The value, $\bar{V}_2^0 = 18.1$ cc., for hydrochloric acid, which is the average of three closely agreeing determina-

tions, yields the secondary reference value, $\bar{V}_i^0 = 18.1$ cc., for the chloride ion. All other ionic volumes were obtained by suitable combinations of this value with the partial molal volumes of salts at infinite dilution. The calculation of \bar{K}_i^0 was similar, but only one value of \bar{K}_2^0 for hydrochloric acid was available. Since \bar{V}_i^0 and \bar{K}_i^0 for a particular ion can often be calculated by several combinations of independent data, the evaluation of these individual ionic properties permits an averaging process which decreases the probable error in the estimation of $\Delta\bar{V}^0$ and $\Delta\bar{K}^0$.

TABLE 4

*The effect of pressure upon the equilibrium constant of the reaction, $\text{H}_2\text{O} \rightleftharpoons \text{H}^+ + \text{OH}^-$, in pure water**

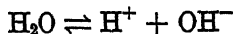
P IN BARS	$K^{(p)}/K$					$\Delta\bar{H}^0$ 25°C.	$\Delta\bar{S}^0$ 25°C.
	5°C.	15°C.	25°C.	35°C.	45°C.		
1	1	1	1	1	1	13.5†	-18.7‡
200	1.24 ₈	1.225	1.202	1.180	1.16 ₈	13.2	-19.4
400	1.54 ₂	1.490	1.435	1.384	1.34 ₅	12.9	-20.1
600	1.8 ₉₈	1.80 ₀	1.703	1.61 ₂	1.54 ₈	12.6	-20.8
800	2.317	2.16 ₂	2.009	1.86 ₈	1.7 ₆₈	12.2	-21.8
1000	2.81 ₆	2.58 ₅	2.358	2.15 ₄	2.0 ₀₉	11.8	-22.8
$\Delta\bar{V}^0$	-26.1	-24.9	-23.4	-21.8	-20.6		

* $\Delta\bar{K}^0 = -52 \times 10^{-4}$ (at 25°C.) used at all temperatures.

† Kilocalories.

‡ Calories.

In illustrating the use of table 3, we have estimated $\Delta\bar{V}^0$ for several ionic equilibria, and calculated $\log K^{(p)}/K$ by equation 12. The results for the important reaction



are shown in table 4 and figure 1. The ionization constant increases with pressure, and the relative increase is the greater the lower the temperature. In fresh water at 0°C. and at a depth corresponding to a pressure of 1000 bars, the ionization constant of water would be about three times its value at the surface. For pure liquid water, the molal volume and its variation with pressure were calculated from density and compressibility data given in the *International Critical Tables*. From table 3, $\bar{V}_{\text{H}^+}^0 + \bar{V}_{\text{OH}^-}^0 = -5.3$ cc. at 25°C. The temperature coefficient of this quantity was estimated by subtracting the temperature coefficient of \bar{V}_2^0 for sodium chloride (8, 16) from the sum of the corresponding values for hydrochloric acid (5) and sodium hydroxide (4). Since the magnitude of $\Delta\bar{V}^0$ could hardly be in

error by more than 1 cc. at the extreme temperatures, its decrease with temperature is unmistakable. On the other hand, the value of $\Delta\bar{K}^0$ could be estimated at 25°C. only, and had to be used at all temperatures. Without attempting to estimate the accuracy of the results, the inadequacy of the data is indicated by limiting the decimal places recorded in table 4.

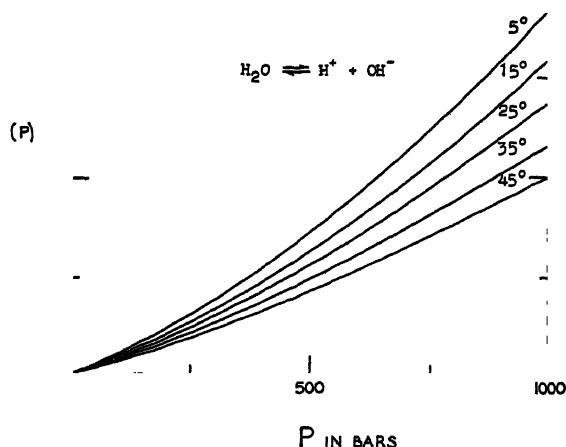


FIG. 1. The effect of pressure upon the ionization of pure water

TABLE 5

The effect of pressure upon ionic equilibria in pure water at 25°C.

REACTION	$\Delta\bar{F}^0$ $P = 1^*$	$10^4\Delta\bar{K}^0$ $P = 1^*$	$K^{(P)}/K$				
			$P = 200^*$	$P = 400^*$	$P = 600^*$	$P = 800^*$	$P = 1000^*$
$\text{CH}_3\text{CO}_2\text{H} \rightleftharpoons \text{H}^+ + \text{CH}_3\text{CO}_2^-$	-9.2	-17	1.075	1.154	1.235	1.32	1.41
$\text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-$	-29	0†	1.26	1.60	2.02	2.6	3.2
$\text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{--}$	-27.8	-87	1.241	1.522	1.85	2.22	2.65
Anhydrite $\rightleftharpoons \text{Ca}^{++} + \text{SO}_4^{--}$	-49.3	-142	1.47	2.12	3.00	4.20	5.80
Calcite $\rightleftharpoons \text{Ca}^{++} + \text{CO}_3^{--}$	-58.3	-157	1.58	2.44	3.69	5.52	8.10
Aragonite $\rightleftharpoons \text{Ca}^{++} + \text{CO}_3^{--}$	-55.3	-157	1.54	2.32	3.44	5.01	7.18
Magnesite $\rightleftharpoons \text{Mg}^{++} + \text{CO}_3^{--}$	-52.3	-169	1.50	2.21	3.18	4.49	6.24
Witherite $\rightleftharpoons \text{Ba}^{++} + \text{CO}_3^{--}$	-61	-185	1.61	2.53	3.88	5.84	8.60

* Total pressure in bars.

† $\Delta\bar{K}^0$ assumed negligible.

The values of $\Delta\bar{H}^0$ and $\Delta\bar{S}^0$ given in table 4 are rough estimates which may be considerably in error at the higher pressures. Nevertheless, it seems safe to conclude that these thermochemical quantities become more negative, the higher the pressure.

Table 5 shows the effect of pressure upon other important ionization and

solubility constants at 25°C. For the solids, the molal volumes and their pressure coefficients were calculated from densities and compressibilities given in the *International Critical Tables*. \bar{V}_2^0 and \bar{K}_2^0 for unionized acetic acid are given in tables 1 and 2. \bar{V}_2^0 is known (7) to be 35 cc. for dissolved carbon dioxide at 25°C., and, assuming that \bar{K}_2^0 for this solute is not much greater than the value for acetic acid, $-\Delta\bar{K}^0 < 10$ for the first step in the ionization of carbonic acid and is neglected.

Note that $\Delta\bar{V}^0$ is negative for all of the reactions in table 5. Rough calculations indicate that $\Delta\bar{V}^0$ for the ionization of acetic acid becomes more positive with increasing temperature. This was also the case with water. Therefore, pressure will presumably decrease the ionization constants of these weak electrolytes at very high temperatures. At 25°C., $\Delta\bar{V}^0$ for the process of solution of sparingly soluble salts is usually large, and negative. It is small and negative for the silver halides; and for some very soluble salts, such as lithium iodide and ammonium nitrate, $\Delta\bar{V}^0$ is small and positive at 25°C. Water of crystallization seldom alters $\Delta\bar{V}^0$ by more than 5 cc. at this temperature.

II. THE SOLVENT MEDIUM IS "SALT WATER" (0.725 MOLAL SODIUM CHLORIDE)

The calculation of the effect of pressure upon ionic equilibria in salt solutions is important because of its bearing upon problems in oceanography. Since the ionic strength of normal sea water (41) is about 0.725,⁶ we shall illustrate the calculation for reactions taking place in a 0.725 molal sodium chloride solution (0.713 normal at 25°C.), hereafter referred to as "salt water".

The first step is to shift the standard state from pure water to salt water so that the activity coefficients of all added solutes become unity at infinite dilution in salt water. The activity of water and the activity coefficient of sodium chloride are also unity in 0.725 *m* sodium chloride solution. As a result of this convention, the thermodynamic constant for a reaction in salt water is equal to the mass action "constant" for the reaction at infinite dilution in this medium. The latter may be determined for the ionization of weak electrolytes by electromotive-force measurements. The thermodynamic constant for the solution of a sparingly soluble electrolyte in salt water is practically equal to the solubility product in salt water, just as in this case the thermodynamic constant and solubility product are nearly identical in pure water. For a more soluble electrolyte, its activity coefficient would have to be considered in pure water, but in salt water the

⁶ This would correspond to a chlorinity of 19.430 ‰, according to the recent calculations of J. Lyman and R. H. Fleming (Sears Foundation: J. Marine Research 3, 134 (1940)).

thermodynamic constant would still be approximately equal to the solubility product, because activity coefficients vary only slightly with concentration at ionic strengths of the order 0.725. In any case, the effect of pressure upon an equilibrium in salt water is given by

$$RT \left(\frac{\partial \ln K^*}{\partial P} \right)_{T,m} = -\Delta \bar{V}^{0*} = - \left[\sum \bar{V}_i^{0*} - \sum \bar{V}_i^{0*} \right] \quad (14)$$

if we let an asterisk indicate the use of this medium in defining the standard state.

The second step is the evaluation of partial molal volumes (\bar{V}_2^{0*}) at infinite dilution in salt water. Wirth's (42, 43) studies of mixed electrolyte solutions yield results from which these values may be directly evaluated for hydrochloric acid and for potassium chloride, potassium bromide, and potassium sulfate. In the absence of further suitable data of the same nature, all other values of \bar{V}_2^{0*} must be estimated from results obtained in simple binary solutions. For such systems, equation 3 leads to the relation,

$$\bar{V}_2 = \bar{V}_2^0 + A_v \sqrt{\Gamma} \quad (15)$$

in which Γ is the ionic concentration ($\sum c_i z_i^2$), and

$$A_v = \frac{(3000 - c \bar{V}_2^0) S_v}{(2000 + c^{3/2} S_v)(\nu z_+ z_-)^{1/2}} \simeq \frac{3/2 S_v}{(\nu z_+ z_-)^{1/2}} \quad (16)$$

The approximate expression for A_v may be used at 0.725 m without introducing an error greater than 0.1 cc. Wirth (43) observed that, below $\Gamma \simeq 1.5$, the presence of sodium chloride in a solution of any one of the three potassium salts affects the partial molal volume of the latter only by its contribution to Γ , the numerical value of A_v remaining unchanged. Upon the *assumption* that this behavior is characteristic of all salts in the presence of sodium chloride, we obtain

$$\bar{V}_2^{0*} = \bar{V}_2^0 + A_v \sqrt{2 \times 0.713} \quad (17)$$

by substituting the value of Γ corresponding to the 0.725 m solution of sodium chloride. This equation will serve as a good approximation, but it is not likely to be strictly valid, even for salts. For hydrochloric acid in sodium chloride solutions it yields a value of \bar{V}_2^{0*} some 0.3 cc. lower than the direct experimental (42) value, 19.6, which is used to evaluate \bar{V}_{Cl}^{0*} , the secondary reference upon which the other ionic volumes in table 6 are based. In the estimation of the latter quantities, A_v was derived from the approximate form of equation 16 and the values of S_v given in table 1.

The third and last step is the representation of \bar{V}_2^{0*} as a function of the pressure, and the integration of equation 14. There appear to be no measurements of compressibilities in solutions of mixed electrolytes which

permit the calculation of \bar{K}_2^{0*} directly. In the preceding paragraphs, two assumptions were introduced which should enable us to approximate this quantity. Thus, the equation

$$\bar{K}_2 = \bar{K}_2^0 + A_K \sqrt{I} \quad (18)$$

in which the parameter A_K is characteristic of a given electrolyte (18), may be assumed applicable in mixtures containing sodium chloride without changing the value of A_K . Accordingly

$$\bar{K}_2^{0*} \simeq \bar{K}_2^0 + A_K \sqrt{2 \times 0.713} \quad (19)$$

TABLE 6

Partial molal volumes and compressibilities at infinite dilution in "salt water" (0.725 m sodium chloride) at 25°C. and 1 atmosphere

COMPOUNDS	\bar{V}_2^0	$10^4 \bar{K}_2^{0*}$	IONS†	\bar{V}_i^0	$10^4 \bar{K}_i^0$
H ₂ O	18.86‡	+6.89‡	H ⁺	0	0
HCl	19.6‡	-7	Na ⁺	-0.6	-37
NaCl	19.0‡	-45	K ⁺	9.7	-32
KCl	29.3‡	-39	NH ₄ ⁺	19.6	
KBr	36.2‡	-30	Mg ⁺⁺	-21	-73
KI	47.3	-16	Ca ⁺⁺	-16.6	-62
KNO ₃	41.1	-26	Ba ⁺⁺	-12.1	-86
KOH	8.4	-71	Cl ⁻	19.6	-7
KHCO ₃	36	-30	Br ⁻	26.4	+2
K ₂ SO ₄	41.4‡	-122	I ⁻	37.5	+16
Na ₂ CO ₃	1.5	-150	OH ⁻	-1	-38
CaCl ₂	22.6	-76	NO ₃ ⁻	31	+6
BaCl ₂	27.1	-100	HCO ₃ ⁻	26	+2
MgCl ₂	18.4		CO ₃ ⁻⁻	2.7	-74
MgSO ₄		-133	SO ₄ ⁻	22	-60

† Some of the ionic quantities are averages obtained from several combinations of data in addition to those given in this table.

‡ These quantities were evaluated directly from experimental data.

in salt water at 25°C. On the other hand, if it be assumed that the effect of 0.725 m sodium chloride upon the compressibility of another electrolyte at infinite dilution in this medium is the same as that of an external pressure equal to the effective pressure ($P_e = 207$ bars) due to the sodium chloride, then

$$\bar{K}_2^{0*} \simeq \bar{K}_2^0 \left[\frac{B+1}{B+P_e} \right]^2 = \bar{K}_2^0 \left[\frac{B+1}{B^*} \right]^2 \quad (20)$$

In the new medium, salt water, $B^* (= B + P_e)$ plays a similar rôle to that of B in pure water. For a 0.725 m sodium chloride solution, $P_e = 207$

bars at 25°C., and $B = 2996$ bars at this temperature (10). The right-hand member of equation 20 is therefore $0.87\bar{K}_2^0$.

Equations 19 and 20 involve assumptions analogous to those upon which equations 17 and 7 are based, but, unlike the latter equations, are not yet subject to experimental verification. Fortunately, \bar{K}_2^{0*} need not be accurately known for most calculations, and alternative values estimated by equations 19 and 20 differ only by 10 to 20 per cent. The provisional estimates of \bar{K}_2^{0*} recorded in table 6 are based upon equation 20.

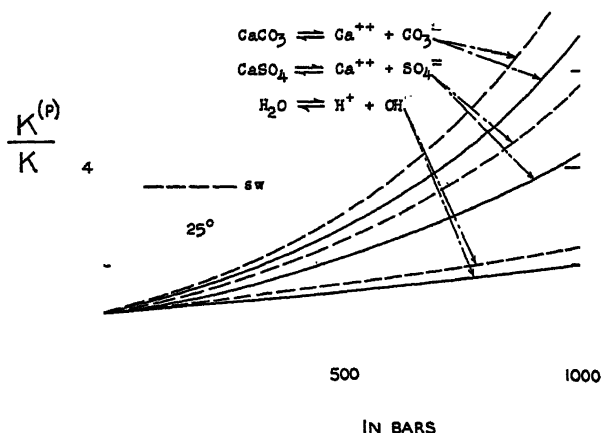


FIG. 2. Comparison of the effects of pressure upon ionic equilibria in pure water (—) and in salt water (---) at 25°C.

According to equation 7, $\bar{K}_2^{0*(P)}$ is given at any pressure by

$$\bar{K}_2^{0*(P)} = \bar{K}_2^{0*} \left[\frac{B^* + 1}{B^* + P} \right]^2 \quad (21)$$

from which we obtain

$$\bar{V}_2^{0*(P)} = \bar{V}_2^{0*} - \bar{K}_2^{0*} \left[\frac{(B^* + 1)(P - 1)}{B^* + P} \right] \quad (22)$$

Combination of this expression with equation 14 leads to

$$RT \ln (K^{*(P)}/K^*) = -\Delta \bar{V}^{0*}(P - 1) + \Delta \bar{K}^{0*} \left[(B^* + 1)(P - 1) - (B^* + 1) \ln \left(\frac{B^* + P}{B^* + 1} \right) \right] \quad (23)$$

Equations 21, 22, and 23 are formally identical with equations 10, 11, and 12 and are presumably valid over the same range of pressures, but,

in view of the assumptions involved in the numerical evaluation of \bar{V}_2^{0*} and \bar{K}_2^{0*} in the absence of adequate experimental data, the accuracy with which pressure effects may now be calculated in salt water is undoubtedly inferior to that of similar calculations in pure water. The effect of pressure upon several ionic equilibria in salt water is shown in figure 2. The results are compared with those previously given for pure water. It is noteworthy that, in every case so far examined, the effect of pressure and the presence of sodium chloride alter the rate of change of the equilibrium constant with pressure in the same direction, a result which may be shown to be in accord with Tammann's hypothesis.

In conclusion, we note that the relatively large factor, RT , which appears in equations 12 and 23, makes it unnecessary to know the volume and compressibility terms with high accuracy if the pressure is less than 1000 atmospheres. Furthermore, this factor is usually more important than changes of $\Delta\bar{V}^0$ with temperature, when comparisons are made at several temperatures.

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THE BINARY SYSTEM CARBON DIOXIDE-WATER UNDER PRESSURE¹

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Data are presented for the binary system carbon dioxide-water between 12° and 100°C. and at pressures up to 700 atmospheres. It is shown that the composition of the phase rich in water above the critical temperature of carbon dioxide can be calculated quite accurately using the equation:

$$\frac{(d \ln N_2)_T}{(dP)} = \frac{V'_2 - V_2}{RT}$$

Sharp minima are found in the composition-pressure isotherms for the phase rich in carbon dioxide.

The present discussion will deal with the equilibrium concentrations of the two phases of carbon dioxide in water (phase rich in water) and water in carbon dioxide (phase rich in carbon dioxide) as functions of pressure and temperature. Sander and others (13, 14, 17, 18) have investigated either one or the other phase. The data given in this article are those of Wiebe and Gaddy, except where specific reference is made to the work of others.

RESULTS

The composition of the phase rich in water (solubility of carbon dioxide in water) as a function of temperature and pressure is given in table 1. The composition *versus* temperature plot shown in figure 1 terminates abruptly at 12°C. above 25 atmospheres. Although several attempts were made to get data in the region above 25 atmospheres and below 12°C., only one series of measurements, at 10°C. and 150 atmospheres, was obtained. The formation of solid, presumably carbon dioxide hexahydrate, in the connecting pipe-lines made further measurements impossible in our appa-

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ratus. The fact that one series of measurements was obtained is very characteristic of the mode of formation of these gas hydrates (15). A special technique, e.g., sudden expansion, must often be used to initiate the formation of the hydrate, although once started it will form readily.

As indicated by the slopes of the solubility curves, the heat of solution changes sign at the higher pressures. The calorimetric data on the heat of solution of carbon dioxide gas in water at atmospheric pressure are as follows: Thompson (16), 5880 calories (18°); Berthelot (4), 5600 calories (18°); Adolph and Henderson (1), 4690 calories (22°).

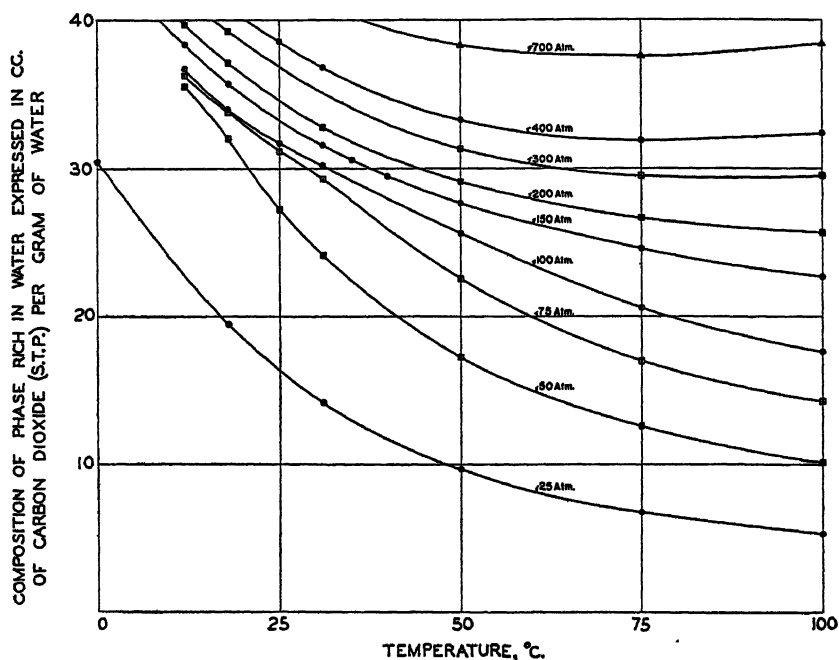


FIG. 1. Plot of composition against temperature

Approximate calculations of the heat of solution from solubility data at 1 atmosphere by means of the equation (6) which holds for ideal solutions,

$$\ln \frac{N'_2}{N_2} = \frac{\Delta H}{R} \left(\frac{T' - T}{TT'} \right) \quad (1)$$

where N'_2 and N_2 are the mole fractions of carbon dioxide at T' and T , respectively, give values similar to the ones above, depending on the temperature interval. For instance, a calculation using Bohr's data (5) for the interval from 0° to 25°C. will give a value of -5270 calories, while a similar one for 25° to 50°C. yields -4250 calories, giving an average of

-4760 calories. This value is close to the one obtained by Adolph and Henderson. The reversal of the trend from negative to positive values of the heat of solution may be explained if we assume that the change of the heat of solution with pressure is due solely to the change of heat content of carbon dioxide, and given by the relation is

$$\left(\frac{dH}{dP}\right)_T = \mu C_p \quad (2)$$

Burnett (6) determined μ experimentally at various temperatures up to 100 atmospheres, and Michels-Veraart (11) calculated the values of C_p .

TABLE 1
Composition of water-rich phase

TOTAL PRESSURE IN ATMOS- PHERES	SOLUBILITY OF CARBON DIOXIDE IN WATER, IN CUBIC CENTIMETERS (AT S.T.P.) PER GRAM OF WATER									
	0°C.	12°C.	18°C.	25°C.	31.04°C.	35°C.	40°C.	50°C.	75°C.	100°C.
25	30.51*		19.51		14.18	12.95	11.62	9.71	6.82	5.37
50		35.54	32.03	27.23	24.15	22.21	20.35	17.25	12.59	10.18
75		36.33	33.85	31.17	29.33	27.84	25.81	22.53	17.04	14.29
100		36.77	33.98	31.75	30.17	29.13	27.81	25.63	20.61	17.67
125							28.71	26.77		
150		38.39	35.75		31.59	30.52	29.39	27.64	24.58	22.73
200		39.77	37.17		32.78	31.83	30.74	29.14	26.66	25.69
300		41.07	39.31					31.34	29.51	29.53
400				38.62	36.78	35.73	34.87	33.29	31.88	32.39
500				39.74	38.67	37.99	36.73			
600								36.73		
700								38.34	37.59	38.50

* Zelvinskiĭ (18).

Since $C_p - C_v$ must be rather large in the neighborhood of the critical region (9), we obtain for $\int_{50}^{100} \mu C_p dP$ at 75°C. approximately 500 calories, whereas the same value calculated from the corresponding heats of solution using equation 1 is in the neighborhood of 800 calories. Considering the assumptions made, this represents a fair check at least of the order of magnitude.

Figure 2 shows a plot of compositions vs. pressure. Using the equation (10)

$$\left(\frac{d \ln N_2}{dP}\right)_T = \frac{V'_2 - \bar{V}_2}{RT} \quad (3)$$

which holds, however, only for ideal solutions and where N_2 is the mole fraction of carbon dioxide in water and V'_2 and \bar{V}_2 are the molar volume

and partial molar volume of carbon dioxide in the phase rich in carbon dioxide and the phase rich in water, respectively, we may calculate the solubility of carbon dioxide from 40° to 100°C. with considerable accuracy. V'_2 was calculated from data of Deming and Deming,³ and \bar{V}_2 was taken to be equal at all pressures and temperatures to the molar volume of carbon dioxide in water at 0°C. and atmospheric pressure (2, 17.). Table 2 gives a comparison between calculated and experimental results.

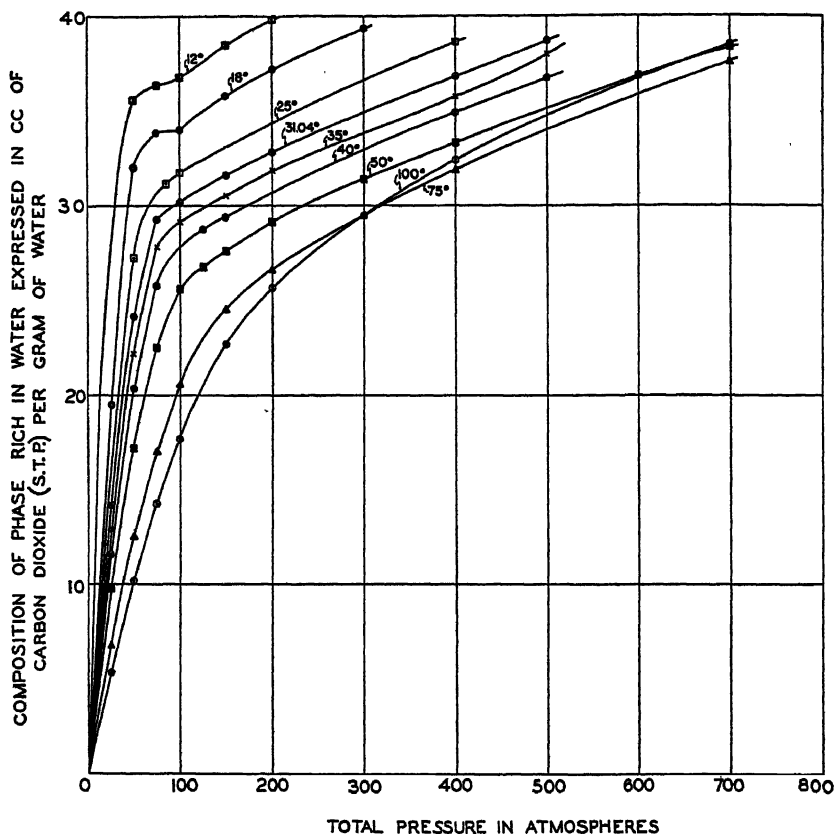


Fig. 2. Plot of composition against pressure

The composition of the phase rich in carbon dioxide as a function of pressure and temperature is given in table 3 and figure 3. For comparison we have plotted the 50°C. isotherm for water vapor in compressed hydrogen from the work of Bartlett (3). The very sudden reversal in the slopes of

³ Dr. and Mrs. Deming kindly gave us permission to use their unpublished data on carbon dioxide, calculated from the work of Michels, Bijl, and Michels (12).

TABLE 2

"PARTIAL PRESSURE"* OF CO ₂ IN ATMOS- PHERES	MOLE FRACTION OF CARBON DIOXIDE IN WATER							
	40°C.		50°C.		75°C.		100°C.	
	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.	Exptl.	Calcd.
50	0.0162	0.0159	0.0138	0.0136	0.0102	0.0099	0.0083	0.0082
75	0.0205	0.0202	0.0179	0.0177	0.0137	0.0134	0.0116	0.0113
100	0.0220	0.0221	0.0203	0.0198	0.0164	0.0160	0.0142	0.0138
150	0.0232	0.0234	0.0219	0.0214	0.0195	0.0191	0.0181	0.0175
200	0.0243	0.0246	0.0230	0.0226	0.0211	0.0208	0.0204	0.0199
300		0.0266	0.0247	0.0247	0.0233	0.0234	0.0234	0.0230
400	0.0275	0.0285	0.0262	0.0266	0.0251	0.0257	0.0256	0.0256
700		0.0338	0.0301	0.0318	0.0294	0.0315	0.0303	0.0322

* Total pressure — vapor pressure of water corrected by means of Poynting's relation. (See Wiebe and Gaddy: J. Am. Chem. Soc. 55, 947 (1933)). Table of values shown in table 2 in Wiebe and Gaddy (J. Am. Chem. Soc. 56, 76 (1934)).

TABLE 3

Composition of the phase rich in carbon dioxide in equilibrium with the phase rich in water

GRAMS OF WATER PER LITER OF EXPANDED GAS AT S.T.P.

IN ATMOSPHERES	25°C.	31.04°C. (critical temperature)	50°C.	75°C.
1	0.023	0.032	0.093	0.242
25	0.00132	0.00183	0.00498	0.00855
50	0.00104	0.00129	0.00308	
60			0.00287	
75			0.00281	
100	0.00267	0.00293	0.00361	0.00666
110				0.00652
125				0.00687
150	0.00289		0.00490	0.00763
200	0.00303	0.00338	0.00544	0.00910
300				
400		0.00383	0.00610	0.01060
450	0.00322			
475	0.00321			
500	0.00319	0.00386		
525		0.00382		
550		0.00384		
600			0.00637	0.01120
700			0.00644	0.01125

the curves is very striking. A consequence of this is that water dissolved in the phase rich in carbon dioxide changes from a "vapor" to a liquid. This may be qualitatively shown by means of the general equation for

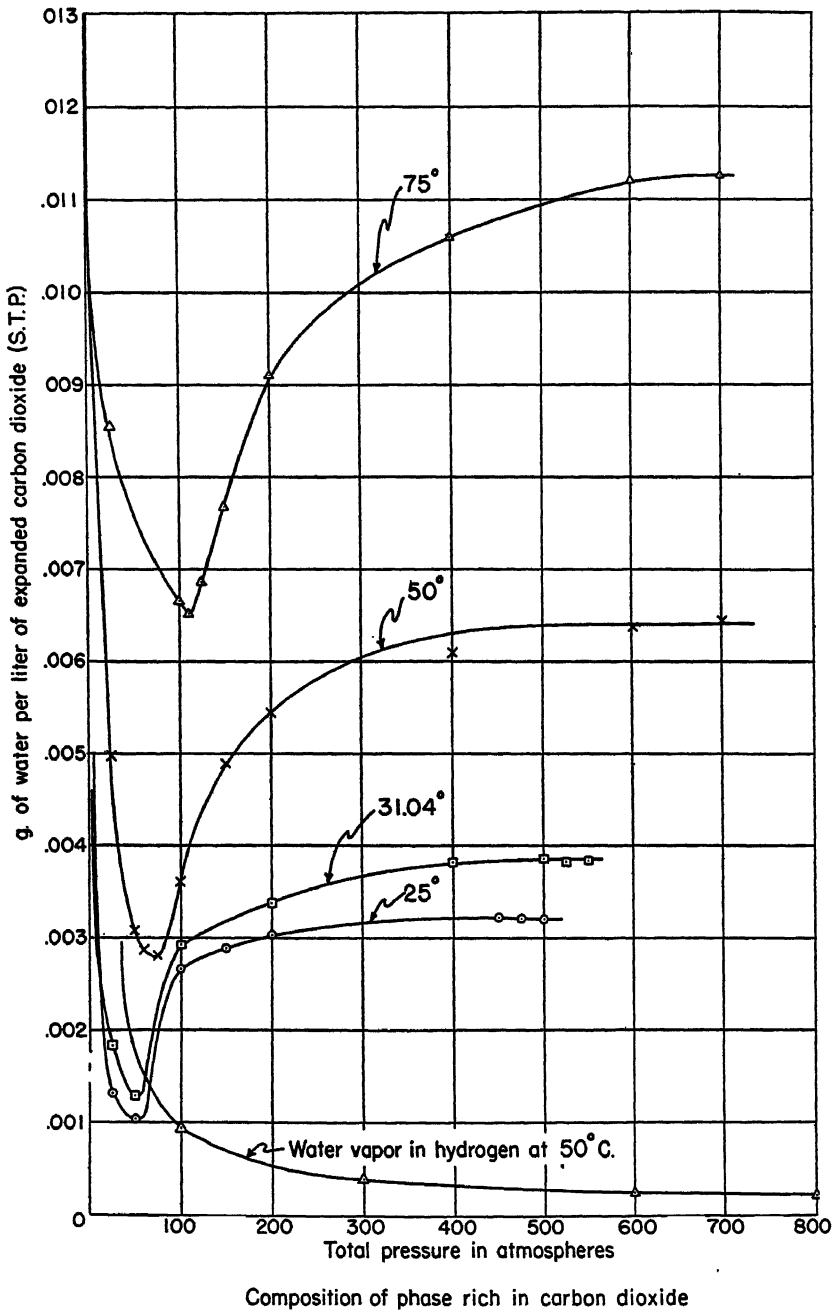


FIG. 3. Composition of the phase rich in carbon dioxide as a function of pressure and temperature

each component of a binary isothermal system with two phases in equilibrium (16):

$$\bar{V}'_1 - \bar{V}_1 = \left(\frac{d\bar{F}_1}{dN_1} \right)_P \frac{dN_1}{dP} - \left(\frac{d\bar{F}'_1}{dN'_1} \right)_P \frac{dN'_1}{dP} \quad (4)$$

where \bar{V}_1 and \bar{V}'_1 are the partial molar volumes of water in the phases rich in water and rich in carbon dioxide, respectively. From the data we know that dN'_1/dP changes from negative to positive, dN_1/dP is positive but approaching some small value or zero, and $d\bar{F}_1/dN_1$ and $d\bar{F}'_1/dN'_1$ are both positive. The left-hand factor of equation 4, $\bar{V}'_1 - \bar{V}_1$, therefore diminishes; in other words, the partial molar volumes of water in the two phases may become practically equal.

At various pressures beyond the ones given in the paper, depending on the temperature, a very sudden increase in the water content of the phase rich in carbon dioxide was noted. The exact composition could not be determined in the apparatus used, and the significance has never been definitely determined. It may have been due to some experimental factor, or it may be a critical mixing phenomenon.

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CHEMISTRY OF COAL HYDROGENATION^{1,2}

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A review is presented of the important variables in the hydrogenation of coal. Catalysts, contact time, temperature, and pressure are the chief topics of the discussion. Volatile halogen compounds and a few amphoteric sulfides—namely, molybdenum and tungsten disulfides and stannous sulfide—are the most active of the coal-hydrogenation catalysts. The halogens and stannous sulfide are reported to be particularly effective in primary liquid-phase hydrogenation; molybdenum and tungsten disulfides are preferred for vapor-phase hydrogenation of tars or of liquid primary coal-hydrogenation products.

The probable mechanism of the coal-hydrogenation reactions is discussed on the basis of results of a study of the temperature coefficients of the rates of consumption of hydrogen, removal of oxygen, and liquefaction of coal. Data are given on the effects of temperature in the range 420–460°C., of contact times from 1.5 to 4 hr., and of pressures from 200 to 300 atmospheres on the yield of oil from coal by hydrogenation.

I. INTRODUCTION

The essential chemical difference between bituminous coal and petroleum is that the carbon-to-hydrogen atomic ratio is about 1.2 for the former and about 0.6 for the latter. In 1913 Dr. F. Bergius discovered that it is possible to add hydrogen to coal at a pressure of about 200 atmospheres and a temperature of about 450°C. Under these conditions most of the oxygen in the coal was hydrogenated to form water, some of the nitrogen to form ammonia, and most of the sulfur to form hydrogen sulfide; enough additional hydrogen atoms were chemically combined with the coal substance to produce a liquid similar to petroleum. Subsequently, the I. G. Farbenindustrie A.-G. discovered contact catalysts that increased the speed

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of the addition of hydrogen to the coal. Gasoline is now being produced on a large scale from coal by hydrogenation in Germany and in Great Britain. In 1939 Germany, with its huge plants at Leuna, Stettin, Gelsenkirchen, Bolen, and Magdeburg, was producing about 1,000,000 tons of such gasoline a year. Great Britain has a large plant at Billingham, erected by the Imperial Chemical Industries, which produces about 150,000 tons of gasoline a year by this method. Similar, though smaller, plants are in operation in Japan, France, and the United States.

II. AGITATION AND VEHICLE

One should expect that, in a heterogeneous reaction such as that between a suspension of coal in oil and hydrogen, agitation would be of paramount importance. Unfortunately, the evidence on this point is both meager and confusing. In experiments at the Bureau of Mines coal-hydrogenation plant (17) and in those at the British Fuel Research Laboratory (3), variations in rate of hydrogen flow by a factor of about 10 produced no appreciable variation in yield of oil. In the systems as set up for these tests the only source of agitation was that produced by hydrogen-gas bubbles rising through a vertical column of coal-oil suspension. However, the results obtained by Morgan and Veryard (31) for hydrogenation of coal and tar under conditions of turbulent flow of the reactants show that the rate of reaction is markedly increased by turbulence. This conclusion should be entertained with the reservation that, as Morgan and Veryard did not use any catalyst, the effect of turbulence may have been largely one of providing a catalyst: namely, the converter walls, by rupture of the adhering oil film on the metal surface.

Recent work by Storch and his coworkers (36) indicates that the rate of consumption of hydrogen is limited by a diffusion process, presumably diffusion of one of the reactants to the contact catalyst surfaces. This work was done in 1.2-liter rotating autoclaves, involving no agitation by gas bubbles; hence it is perhaps illogical to compare this system with that in the continuous experimental plant (17). If, however, one assumes that neither the mechanical agitation in the rotating autoclave nor the agitation by gas bubbling in the continuous plant is capable of disturbing the liquid film on the catalyst surfaces, then relatively large changes in the amount of such agitation would not affect the rate of the contact catalytic hydrogenation reaction, provided no turbulence were produced.

The effects produced in coal hydrogenation by adding a vehicle (liquid suspension medium) and catalysts to the coal were studied by Horton, King, and Williams (19) and by Warren, Bowles, and Gilmore (41). The data show that the separate effects of catalyst and vehicle are not additive. With small amounts of catalyst the addition of a vehicle results in an ap-

preciably enhanced yield; however, with large amounts of catalyst (that is, greater than about 0.5 per cent), the effect of the addition of vehicle is small and may even become negative. The two variables,—amounts of catalyst and of vehicle,—are therefore not independent of each other. This is probably due to the primary liquid product serving as vehicle for the remainder of the coal substance. Hence if a large proportion of catalyst is present, the production of vehicle is rapid enough to result in a yield of oil that equals or is even greater than that obtained in the presence, at the start, of both vehicle and catalyst. The vehicle produced from the coal itself is probably a more efficient solvent than any of the other vehicles employed by the experimenters listed above (19, 41).

TABLE 1

Experiments on wall catalysis with 2-liter converter

Beamshaw coal, low-temperature tar vehicle; contact time, 2 hr.; temperature, 450°C.; initial pressure, 100 atmospheres of hydrogen

EXPERIMENT NO.	LINER FITTED	CATALYST	INSOLUBLE RESIDUE (PER CENT OF DRY ASH-FREE COAL)
1	No	None	12.0
2	Yes	None	45.0
3	No	0.1 per cent $\text{Sn}(\text{OH})_2$	9.8
4	Yes	0.1 per cent $\text{Sn}(\text{OH})_2$	14.5
5	No	50 per cent Pyrex glass	15.3

III. CATALYSTS

The catalytic behavior of the converter walls has been investigated by the British Fuel Research Laboratory (6) and found to have considerable importance. These experiments were conducted in a 2-liter autoclave, using a Pyrex-glass "liner." The results are given in table 1.

Experiment 5 shows that Pyrex glass has very little effect; hence, conclusions concerning the catalytic effects of the converter walls and of the stannous hydroxide are sound. In present industrial coal-hydrogenation practice the converters are about 1 meter in diameter; hence catalysis by the converter walls may have less importance than is indicated in table 1. It should, however, be remembered that in engineering development, work such as was done by Vallette (39) in France and recently by Morgan and Veryard (31) in Great Britain, where very much narrower tubes were used than those in the experiments of table 1, the catalytic effect of the converter walls has considerable importance. One might suppose that the activity of the converter walls would be rapidly decreased by poisoning. Such is not the case, as was shown by experiments in the British Fuel Research Laboratory (4). The only way in which converter-wall activity

could be avoided was by the use of a glass liner. Indeed, one patent (10) specifies periodic activation of the converter walls by the action of small amounts of iodine in the hydrogenation of pitch and coal. No addition of iodine or its compounds is made to the reaction mixture. Additional data indicating the importance of the catalytic activity of the converter walls are reported in connection with some laboratory experiments on tar hydrogenation (27). These results also indicate that the walls of the autoclave have considerable influence on the hydrogenation process.

The results of Warren, Bowles, and Gilmore (41), who used stannous oxide in the absence of a vehicle, showed that the yield of volatile products is an approximately linear function of the logarithm of the per cent of catalyst used. This relationship holds until about 1 per cent of stannous oxide is present; for larger percentages the increase in yield due to additional catalyst is smaller than would be expected. In the presence of a vehicle the relationship between yield and amount of catalyst is such that the yield increases much more slowly than the logarithm of the per cent of catalyst used.

The nature of the catalysts employed in coal hydrogenation has been the subject of numerous patents. The presence of appreciable quantities of sulfur compounds and organic substances of high boiling points makes impractical the use of the well-known active hydrogenation catalysts, such as reduced nickel. Moreover, the latter frequently show a negative temperature coefficient⁴ at temperatures of 400° to 500°C. in hydrogenation reactions where sulfur and high-boiling organic substances are absent; because of this, they are not as active as other catalysts that are inferior to them at lower temperatures (1).

The catalysts mentioned in numerous recent patents concerning the destructive hydrogenation of coal and other carbonaceous materials may, for convenience, be classified in four groups as follows: (1) Volatile halides or compounds that will yield volatile halides upon decomposition at process temperatures (42); (2) promoted heavy-metal sulfide and oxide catalysts (43); (3) finely divided metals (44); (4) alkali and alkaline-earth metals and their compounds, plus finely divided metals (45).

The recent patent literature shows the outstanding importance of volatile halogens as catalysts in the primary liquefaction of coal by hydrogenation. This fact may be correlated with the ability of halogens, alkyl halides, and

⁴ The term "negative temperature coefficient" is used to indicate a decreasing rate of reaction with increasing temperature; or, as used in that section of this paper on the solubility of hydrogen, it indicates a decreasing concentration of hydrogen with increasing temperature. These "negative" coefficients as nominally measured are, of course, ratios the value of which is less than unity, as compared with the "positive" coefficients which are greater than unity.

halogen acids to function as catalysts in the thermal decomposition of such oxygenated organic compounds as ethers and aldehydes (11, 16).

A few amphoteric sulfides—namely, molybdenum and tungsten disulfides and stannous sulfide—are the most active of the metal sulfide catalysts. Stannous sulfide is reported to be particularly effective in the primary liquid-phase hydrogenation, whereas molybdenum and tungsten disulfides are preferred for vapor-phase hydrogenation of tars or liquid primary coal-hydrogenation products. The metal sulfides are, in general, more active than the oxides, and it is desirable to maintain a minimum partial pressure of about 1 atmosphere of hydrogen sulfide to avoid conversion of metal sulfides to oxides or to metals. The metalloids phosphorus, arsenic, selenium, and tellurium, or their compounds, are reported to be desirable additions to liquid-phase coal-hydrogenation catalysts (22).

The results obtained from experiments with various catalysts for the liquid-phase hydrogenation of tar are in general very similar to those for the liquid-phase hydrogenation of coal. An extensive survey (8) was made in the British Fuel Research Laboratories, using for each experiment 250 g. of topped low-temperature tar, a 2-liter rotating autoclave, an initial (cold) hydrogen pressure of 100 atmospheres, a temperature of 450°C., a reaction time of 2 hr., and 5 per cent of powdered catalyst. The halogens were found to have outstandingly high catalytic activity, iodine being more active than bromine or chlorine. The hydrogen halides and volatile organic halides gave results similar to those of the halogens. Stannic oxide was a good catalyst, but stannous chloride was much superior. The power possessed by sulfur of activating other substances, or perhaps acting catalytically in the presence of other substances, was found to be most marked with molybdic acid. Thus, while 2.5 per cent of molybdic acid gave a tarry product containing 23.4 per cent of pitch, a mixture of 2.5 per cent each of sulfur and molybdic acid yielded a light amber-colored oil that was completely soluble in petroleum ether and contained only traces of tar acids and bases. The effect of sulfur was also very marked when it was used with tungstic oxide. The latter is, *per se*, inactive, but a mixture of equal parts of tungstic oxide and sulfur was an active catalyst. Iron, nickel, cobalt, and chromium oxides, which were only slightly active, showed some improvement when mixed with sulfur, whereas the cadmium, tin, and vanadium oxides (which are mildly active catalysts) showed no improvement.

Most of the published information on catalysts for vapor-phase hydrogenation concerns low-temperature tar rather than oils obtained from coal by hydrogenation. A considerable amount of experimental work published in England (8, 24) and Japan (2) on the hydrogenation of low-temperature tar shows that molybdenum oxide supported on alumina gel is a satisfactory

vapor-phase catalyst when used at 480–500°C. and a pressure of 200 to 300 atmospheres of hydrogen. It deteriorates at a relatively slow rate and can be reactivated by oxidation with air. However, for partly hydrogenated middle oils that are free from asphaltic and bitumen-like materials, much more active catalysts, such as pelleted molybdenum and tungsten disulfides, may be used. These catalysts may be prepared by precipitation of the sulfide by acidification of an ammonium thiomolybdate (or thio-tungstate) solution, followed by washing, drying, and pelleting of the powdered trisulfide and subsequent reduction to the disulfide. They are extraordinarily active for vapor-phase hydrogenation, some hydrogenation being effected by them at as low a temperature as 250°C., although 380–400°C. is the practical operating temperature (24). In addition to these catalysts being much more active than molybdenum oxide supported on alumina gel, the composition of the gasoline products obtained by the hydrogenation of middle oils (from coal hydrogenation) with pelleted

TABLE 2
Gasolines produced from middle oils by different vapor-phase catalysts

PER CENT BY WEIGHT	ALUMINA-GEL-SUPPORTED CATALYST	PELLETED CATALYST
Aromatic hydrocarbons.....	27	2
Naphthenic hydrocarbons.....	66	50
Paraffinic hydrocarbons.....	7	48

molybdenum disulfide is characteristically different (7, 15), as shown in table 2.

The enormous differences in the results obtained with these catalysts cannot be due entirely to the fact that one is initially molybdic oxide and the other the disulfide, for it is probable that the oxide catalyst is at least partly converted to sulfide during operation. An x-ray study of these two catalysts would probably be profitable.

It appears desirable to maintain a minimum partial pressure of about 1 atmosphere of hydrogen sulfide to avoid conversion of metal sulfides to oxides or metals (20, 21). The work of Varga and Makray (40) shows that hydrogen sulfide, when used along with a molybdenum catalyst, promotes the hydrogenation of naphthalene, *m*-cresol, and tar oils in general. Hydrogen selenide has a selective effect when used with a molybdenum catalyst, in that it promotes the hydrogenation of naphthalene more energetically than does hydrogen sulfide, and retards the hydrogenation of *m*-cresol and tar acids in general.

The experiments of Hollings, Bruce, and Griffith (18) on the influence of silica as a promoter for molybdic acid in the hydrogenation of tars showed

that, when the yield under a given set of conditions was plotted against the atom percentage of silicon, a curve was obtained containing two maxima at 3.0 and 5.5 per cent and a minimum at 4.5 per cent. These authors also studied the adsorption, at temperatures up to 450°C., of hydrogen, benzene, hexane, and cyclohexane by molybdic acid containing varying amounts of silica. An inspection of the data given for the rate of activated adsorption of hydrogen shows that large decreases in the energy of activation occur between 0.0 and 3.0 atom per cent silicon and between 4.4 and 5.5 per cent, whereas this energy is virtually constant between 3.0 and 4.4 per cent. If one assumes a steadily increasing retardation by coal tar or its hydrogenation products with increasing atom percentage of silicon, it is reasonable to predict on the basis of the changes in energy of activated adsorption of hydrogen precisely the form of curve actually obtained; that is, two maxima, one somewhere between 0.0 and 3.0 per cent silicon and the other between 4.4 and 5.5 per cent.

The efficiency of various promoters in increasing the catalytic activity of molybdenum oxide in the catalytic hydrogenation of phenol to benzene was studied by Kingman and Rideal (25, 5). It was found that a very effective method of introducing the promoter was to prepare the catalyst from the appropriate heteropoly acid, with the promoter as the central atom in the complex. The ammonium salts of the acids were used, and the catalysts were treated with hydrogen for 6 hr. at 480°C. before testing. Of the four catalysts prepared from $(\text{NH}_4)_n[\text{X}(\text{Mo}_2\text{O}_7)_6]$, where X = phosphorus, silicon, tin, or thorium, those containing phosphorus and silicon were much more active than molybdenum oxide, whereas those containing tin and thorium were appreciably less active than molybdenum oxide. The three catalysts prepared from $(\text{NH}_4)_n[\text{M}(\text{MoO}_4)_6]$, containing nickel, chromium, and copper, were much more active than molybdenum oxide. X-ray analysis of the first group of catalysts showed an extremely small particle size for the phosphorus- and silicon-promoted catalysts, the structure of the phosphorus catalysts being entirely different from that of pure molybdenum dioxide. The thorium-promoted catalyst is more crystalline than that promoted with phosphorus, and both the thorium- and the tin-promoted catalysts have structures similar to that of pure molybdenum dioxide. The efficiency of a promoter in the first group of catalysts may therefore depend on prevention of the sintering of nearly amorphous catalysts. X-ray analysis of the second group shows a fairly well developed crystalline structure similar to that of pure molybdenum dioxide; hence, the high activity of these catalysts may be due to an effect different from that for the phosphorus- and silicon-promoted catalysts of the first group. This effect may be distortion of the space lattice caused by introduction of the promoter. The relative surface areas of some of the catalysts were

measured, and it was found that, while the more active catalysts presented larger areas, the variations in surface areas were not directly proportional to the measured differences in catalytic activity. However, inasmuch as it is known that in heat sintering the activity of some catalysts decreases manifold faster than the surface area, this lack of proportionality is not necessarily indicative of a new or qualitatively different phenomenon.

A detailed study of promoters for the dehydrogenation of hexane and the hydrogenation of phenol was published recently by Griffith (12, 13). The optimum promoter concentration for the decomposition of hexane at 500°C., using a molybdenum oxide catalyst, is found to be 4.3 atom per cent for sodium, chromium, cerium, aluminum, barium, boron, and thorium, and 2.2 atom per cent for iron, copper, and lead. The metal oxides of the first group are not reducible under the operating conditions, whereas iron, copper, and lead oxides are reduced to the respective metals. For the hydrogenation of phenol at 400°C., the optimum promoter concentrations for a molybdenum oxide catalyst were 16 atom per cent for cerium, 16.5 for aluminum, and 15.0 for silicon. No adsorption measurements for phenol or hydrogen are reported, and until further data are available it will be difficult to determine the significance of these optimum promoter concentrations.

The same author also presents a series of determinations of dehydrogenation rate for decahydronaphthalene, decane, hexane, and cyclohexane, using chromic oxide gel plus various amounts of magnesia (13, 14). For decahydronaphthalene the activity is constant from 0.0 to 90.0 per cent magnesia and then drops off linearly with increasing content of magnesia. For decane the constant activity range is 0.0 to 50.0 per cent and for hexane 0.0 to 20.0 per cent; for cyclohexane the activity drops continuously with increasing content of magnesia. Griffith explains these data by assuming that the larger hydrocarbon molecules cover many active centers upon adsorption, and hence the dilution effect due to the inert carrier magnesia is not apparent until the active centers are farther apart than the distance necessary for activation and reaction. Similar experiments with a chromic oxide-silica catalyst and addition of various amounts of magnesia show an immediate dilution effect with all hydrocarbons. Griffith concludes from this that chromic oxide promoted with silica presents a surface that differs qualitatively from unpromoted chromic oxide and is not merely one with a larger number of active centers.

Unquestionably this research will be of importance in developing the chemistry of vapor-phase hydrogenation. It would, however, be desirable to have catalytic rate studies on the destructive hydrogenation of larger molecules. There is also a dearth of rate measurements on destructive hydrogenation in the liquid phase of pure compounds of high molecular

weight. Where relatively large molecules are involved, it seems probable that adsorption of such molecules will, beyond a certain limit, retard destructive hydrogenation reactions. Especially in liquid-phase hydrogenation of large molecules, one would expect the rate to be determined largely by that of the activated adsorption of hydrogen on a surface which is almost completely covered by the other reactant. It would, perhaps, be more logical to search for hydrogenation catalysts that do not strongly adsorb the organic molecule to be hydrogenated destructively, or for hydrogenation catalysts that are soluble in the liquids to be hydrogenated, thus avoiding the necessity of a contact catalytic reaction. Tetrahydronaphthalene probably functions in part as a liquid-phase catalyst for coal hydrogenation, and it is likely that the desirability of compounds such as methylnaphthalene, phenols, quinoline, etc., in liquid-phase coal-hydrogenation systems may also be a matter of labile hydrogen atoms in these molecules, in addition to their well-known solvent action on coal. Observations have been reported which indicate that aromatic amines may be regarded as catalysts for the dehydrogenation of glyceraldehyde (37).

IV. TEMPERATURE AND PRESSURE

It is commonly supposed that one of the important reasons for the necessity of a temperature above 400°C. for the liquid-phase hydrogenation of coal is the fact that active pyrolysis of coal does not occur until such temperatures are employed. This explanation probably is too simple, for other factors are involved, such as the positive temperature coefficient of the solubility of hydrogen in most liquids, the decrease in retardation of the reaction rate at high temperatures, owing to decreased absorption of bituminous materials on the contact catalyst, and the effect of temperature and particle size on the rate of depolymerization and solution of the coal substances.

Maxted and Moon (32) showed that the temperature coefficient of the molar solubility of hydrogen in all the liquids tested by them is positive. With a given gas-liquid interfacial area (which is largely determined by the rate and manner of recirculation of hydrogen) both the rate and the total equilibrium amount of hydrogen dissolved by a given weight of liquid will increase with the temperature.

Körösy (26) plotted the critical temperature of gases against the logarithm of the molar solubility. He obtained a straight line for all solvents, but found that hydrogen exhibits anomalous behavior in that its solubility is much higher than would be predicted from its critical temperature. Gases with critical temperatures above 180°A. (-93°C.) have negative temperature coefficients of solubility in liquids, and those with

lower critical temperatures (such as hydrogen, helium, neon, nitrogen, carbon monoxide, argon, oxygen, and nitric oxide) have positive temperature coefficients in most solvents except water, which, at about room temperature, shows a negative coefficient for all gases. This is due to the fact that most solvents have appreciable expansion coefficients at room temperature, whereas that for water is very low. The temperature coefficient of solubility at constant volume of solution is negative for all gases and all solvents. Experiments made in the British Fuel Research Laboratories (9) also showed that hydrogen has a positive temperature coefficient of solubility in hydrocarbon solvents and yielded the results given in table 3 for the solubility of hydrogen and a mixture of hydrocarbons (mainly methane plus ethane) in several oils.

TABLE 3

Solubility of hydrogen and gaseous hydrocarbons in products of tar hydrogenation

SOLVENT	SOLUBILITY AT 20°C., IN VOLUMES OF GAS PER UNIT VOLUME OF SOLVENT PER 1 ATMOSPHERE PARTIAL PRESSURE	
	Hydrogen	Hydrocarbons
Product of hydrogenation of low-temperature tar..	0.065	0.7
Fraction of product boiling to 200°C.....	0.090	0.9
Fraction of product boiling from 200° to 300°C.....	0.050	0.6

TABLE 4

Temperature coefficient of solubility of hydrogen in a middle oil at 200 atmospheres pressure

Temperature, °C.....	25	100	200	300	350
Hydrogen per kilogram of oil, liters....	13	18	28	37	39

The temperature coefficient of the solubility of hydrogen in a middle oil is indicated by the data (34) of table 4. The heat of solution of the hydrogen calculated from these data ranges from about 9500 calories for the 25–100°C. interval to 16,000 calories for the 200–300°C. interval. The variation may be due to failure to reach equilibrium.

Some data on the kinetics of the primary liquefaction of coal by hydrogenation have recently been obtained by H. H. Storch and his coworkers (36). This work was done in a rotating 1200-cc. bomb, using equal weights (100 g. of each) of tetralin and coal plus 1 per cent of tin sulfide and an initial (cold) pressure of 1000 pounds of hydrogen per square inch. The results of this work indicate the following mechanism for the primary liquefaction of bituminous coal by hydrogenation:

(1) The curves of hydrogen consumption vs. time in the temperature

range 370–430°C. are virtually straight lines the slope of which shows a temperature coefficient of 1.20 per 10°C. The low temperature coefficient indicates that the slow step is probably a diffusion process, such as the transport of hydrogen through a liquid film covering the catalyst surfaces. The rates of all of the reactions mentioned in paragraphs 3, 4, and 5 below are independent of the rate of hydrogen consumption.

(2) Below 370°C. the extent of coal liquefaction (as measured by solubility in acetone and benzol) depends on depolymerization by the solvent. The activation energy of this reaction is about 7 kg.-cal., as calculated in the usual way from the temperature coefficient.

(3) Above 400°C. a fast reaction with an activation energy of about 65 kg.-cal. occurs; this reaction is probably the thermal decomposition of the coal substance. It is accompanied by rapid elimination of about 60 per cent of the oxygen of the coal and by absorption of only a negligible quantity of hydrogen.

(4) A second reaction can be clearly differentiated at temperatures above 370°C.; it proceeds at a measurable rate at 400°C., with an activation energy of about 40 kg.-cal. This reaction is slower than that described in paragraph 3 and is apparently also thermal in nature, since the rate is not changed when a glass liner and no catalyst are used. A large consumption of hydrogen occurs concomitant with this reaction, but the slowest or rate-determining step does not involve hydrogen. This step may be the reaction between the products of the thermal dissociation of coal and tetrahydronaphthalene.

(5) At all temperatures above 370°C. there is a condensation of free radicals or unsaturated compounds (generated by the thermal decomposition of the coal substance) into materials more stable than the original coal substance. This new polymer dehydrogenates at temperatures above about 450°C. at a rapid enough rate so that, despite the presence of hydrogen under pressure and of hydrogenation catalysts, some coke is formed. This condensation reaction obscures the kinetics of the reactions mentioned in paragraphs 3 and 4 and results in apparent negative temperature coefficients for the liquefaction and oxygen-removal reactions at temperatures above 400°C.

The above outline of the probable mechanism of coal hydrogenation indicates that the chief function of catalytic hydrogenation is to saturate the free radicals or unsaturated compounds provided by the thermal decomposition of the coal and to hydrogenate carbon to oxygen and double bonds in the later stages of the liquefaction. The kinetics of these hydrogenation reactions is largely obscured by the variety of other reactions proceeding at the same time. Additional experiments similar to those by Storch and his coworkers (36) have been done with a glass liner and in the

absence of catalysts (unpublished work by Storch and others). The results of such work show that, while the rate of hydrogen consumption is markedly increased by the presence of catalysts, the rates of liquefaction and oxygen elimination are not greatly affected by catalysts.

Morikawa (29, 30) and his associates have studied the effect of water vapor and of stannous chloride on the hydrogenation of coal in a 1-liter rotating autoclave. Their results indicate that the primary step of coal liquefaction occurs at about 300°C. and higher. This step is the production of bitumen upon fusion of the coal substance at about 340°C. It is accelerated by the presence of 5 to 20 per cent of water, and is profoundly affected by prolonged heating at low temperatures. The rate of production of normally gaseous hydrocarbons is but slightly affected by the presence of a catalyst such as stannous chloride; Morikawa and his associates state that the primary coal-liquefaction reaction may be the change occurring inside the polymer molecules of the coal substance and may have little to do with the side chains, which yield gaseous hydrocarbons upon hydrogenation. These authors believe that the primary reaction of coal liquefaction is depolymerization of the coal substance, involving some hydrogenation and deoxygenation but with little cracking (rupture of normal carbon-to-carbon valence bonds).

Tests on the effect of pressure, temperature, and contact time were made at the British Fuel Research Laboratory (3) in a small-scale liquid-phase continuous plant having a capacity of about 35 pounds of coal per day. Pressure variations were from 180 to 250 atmospheres; the effect was very small in this pressure range. The effect of increasing the paste input was to increase the heavy-oil product (boiling point, above 310°C.) from zero at 1.2 pounds per hour to 22.9 per cent at 2.2 pounds per hour. The temperature range studied was 420° to 460°C. The yield of insoluble residues was almost constant throughout, in contrast to the results of Hirst and his coworkers (17), given in table 5. The yield of heavy oil decreased with temperature until it vanished at 460°C. Although some increase in gasoline (boiling point, below 200°C.) and middle oil (boiling point, 200–310°C.) accompanied this, there was also a large increase in the formation of gas.

The effect of temperature in the liquid-phase continuous hydrogenation of Pittsburgh-bed coal, calculated from the results obtained by Hirst and his coworkers (17), is shown in table 5.

The fraction of unreacted ash-free coal decreases markedly with the temperature between 420° and 460°C., but the increased liquefaction does not result in an increased yield of oil; the latter decreases, in fact, owing to a rapid increase in the amount of normally gaseous hydrocarbons produced. The effect of varying the contact time, as calculated from Hirst's

data (17), is shown in table 6. The increase in carbonaceous insoluble residue and decrease in yield of gaseous hydrocarbons, with decreasing contact time, is accompanied by a slow increase in yield of oil. The latter, however, reaches a maximum for contact times of about 1.6 hr. and then decreases for shorter times. This maximum is due to the fact that, for any given type of molecule, solubility in benzene is determined largely by molecular weight. When the contact time is too brief to reduce the molec-

TABLE 5

Effect of temperature on hydrogenation of Pittsburgh-bed coal

Contact time, 2 hr.; catalyst, 0.5 per cent SnS + 0.5 per cent MoO₃; pressure, 200 atmospheres; all figures in per cent of moisture- and ash-free coal

TEMPERATURE °C.	ASH-FREE BENZENE- INSOLUBLE RESIDUE	BENZENE-SOLUBLE OILS	NORMALLY GASEOUS HYDROCARBONS	HYDROGEN ABSORBED
420	16.3	73.0	10.4	5.8
433	11.3	71.0	20.7	6.1
447	7.2	67.0	35.2	7.7
459	6.3	57.0	39.8	7.8

TABLE 6

Effect of contact time on hydrogenation of Pittsburgh-bed coal

Temperature, 440°C.; pressure 200 atmospheres; catalyst, 0.5 per cent SnS + 0.5 per cent MoO₃; all figures in per cent of moisture- and ash-free coal

CONTACT TIME* hours	ASH-FREE BENZENE- INSOLUBLE RESIDUE	BENZENE-SOLUBLE OILS	NORMALLY GASEOUS HYDROCARBONS	HYDROGEN ABSORBED
4.0	0.0	72.0	33.8	12.5
2.0	3.0	74.0	21.8	6.9
1.80	6.8	77.0	18.0	7.4
1.64	7.9	77.5	16.0	5.7
1.50	13.4	68.5	14.6	6.2

* This time is the number of hours required to pump one converter volume of paste. The heavy-oil slurry containing the unreacted coal was at the reaction temperature for about 1.5 times the indicated contact time, owing to the continuous evaporation and removal of low-boiling products in the stream of hydrogen passing through the converter.

ular weight below a certain limit, the yield of oil, as measured by solubility in benzene, must decrease. This explanation also accounts for the somewhat abrupt increase in benzene insolubles for a contact time of less than about 1.6 hr.

Pressures of at least 200 atmospheres of hydrogen are found to be desirable in liquid-phase hydrogenation of coal at temperatures of about 450°C. At appreciably lower pressures repolymerization occurs and coke

is formed. The work of Pott and Broche (35) showed that 80 to 90 per cent of the carbonaceous material in bituminous and sub-bituminous coals is rapidly dissolved by tetralin-phenol mixtures at 390–410° C. under the pressure created by the reaction mixture, i.e., with no added hydrogen. The equilibrium partial pressure of hydrogen for the tetrahydronaphthalene-naphthalene equilibrium at 450°C. is reported to be about 10 atmospheres (28). Hence it is probable that hydrogen pressures greater than 10 atmospheres are not thermodynamically necessary for the primary liquefaction of coal. Two other possible functions of high-pressure hydrogen are (1) to increase the amount of dissolved hydrogen, and (2) to accelerate the regeneration of hydroaromatic compounds, such as tetrahydronaphthalene, which serve as hydrogen carriers. It is probable that in the liquid-phase hydrogenation of coal the saturation of the unsaturated molecules produced by the thermal decomposition of the coal is accomplished mainly by reaction with hydroaromatic compounds, and to only a very minor extent by reaction with dissolved or catalytically adsorbed

TABLE 7
Effect of pressure in liquid-phase hydrogenation of coal

PRESSURE		PRODUCTS		
Initial	Maximum	Solids	Water	Oil
<i>atmospheres</i>	<i>atmospheres</i>			
80	203	41.2		37.2
100	245	26.1	6.1	50.2
120	283	21.6	6.4	56.7

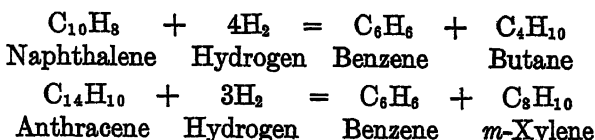
hydrogen. Hence the chief function of high hydrogen pressures and of catalysts is probably the rapid regeneration of hydroaromatics. The slow step in such regeneration may be the diffusion of one or both reactants to the catalyst surfaces.

As an example of the practical importance of high pressures in liquid-phase coal hydrogenation, Pier (33) cites the following: "... the conversion of old coals or of asphalts of high molecular weight which goes but slowly at 200 to 300 atmospheres, but proceeds without difficulty and at a satisfactory rate for large scale production at considerably higher pressures, e.g. 700 atmospheres or more."

The results of experiments by King (23), given in table 7, show the effect of pressure on the hydrogenation of Beamshaw coal in small autoclaves at 445°C. in the absence of a catalyst.

In the vapor-phase hydrogenation of oils obtained from the hydrogenation of coal or coal tar in the liquid phase, hydrogen at high pressure is essential in order to avoid condensation reactions leading to the formation

of coke. It is, however, not known whether the necessity for high pressures of hydrogen is of thermodynamic or kinetic origin. Destructive hydrogenation reactions may be written and their equilibrium relationships calculated from specific-heat data, which indicate that hydrogen pressures above 10 to 50 atmospheres are not thermodynamically necessary. Thus, for example, Sweeny and Voorhies (38) write the reactions:



and calculate the equilibrium constant-temperature-pressure relationships from low-temperature specific-heat measurements. Their calculations show that in the temperature range 450–480°C. a pressure of hydrogen of approximately 10 atmospheres is sufficient to obtain a commercially satisfactory yield (about 50 per cent conversion per pass), assuming that the reaction rates are large enough so that equilibrium may be reached in one pass at a reasonable space velocity. However, much higher hydrogen pressures,—namely, 200 to 500 atmospheres—are found desirable in vapor-phase hydrogenation of oils. It is possible that much higher temperatures exist on the surfaces of the catalysts than in the gas phase, owing to the net exothermicity of the oil-hydrogenation reactions, and at such higher temperatures correspondingly greater pressures of hydrogen would be necessary for a satisfactory yield. It is, however, more probable that the desirability of a pressure of 200 to 500 atmospheres of hydrogen is determined by reaction rates at the catalyst surface, rather than by thermodynamic relationships.

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SOME PECULIARITIES IN THE P - T BORDER CURVES OF MIXTURES OF HYDROGEN AND A PETROLEUM NAPHTHA¹

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The solubility of hydrogen in a volatile petroleum naphtha has been investigated over a temperature and pressure range which included the critical region of the hydrogen-naphtha mixtures. At concentrations of hydrogen below about 18 cc. (at S.T.P.) per gram of naphtha the shapes of the P - T border curves, derived from the solubility data, are such as to suggest the existence of a region of isobaric retrograde condensation at temperatures relatively far removed from the critical region of the mixture. Additional evidence, in support of the suggestion that this behavior is a characteristic property of a general class of mixtures composed of a liquid and a slightly soluble gas, is provided by data given in the literature on the solubility of hydrogen and nitrogen in liquid ammonia.

In the course of an investigation of the solubility of hydrogen in a volatile naphtha, the P - T border curves of several mixtures of the naphtha and hydrogen were determined over a temperature range which included the critical region of the mixture. Within certain limits of hydrogen concentration the shapes of these curves are such as to suggest the existence of a region of isobaric retrograde condensation at temperatures far removed from the critical region of the mixture. As far as can be learned from the literature, the existence of such a region has not been pointed out before. The present report gives a summary of the experimental results on the hydrogen-naphtha mixtures as well as a discussion of the results on other mixtures reported in the literature which appear to have similar properties.

I. EXPERIMENTAL

The apparatus and experimental procedure used for determining the P - T border curves of a mixture have been fully described in previous papers (2, 3).

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The naphtha was a narrow-boiling fraction composed principally of the pentanes and hexanes. Its composition as determined by distillation, as well as data on other physical properties, are given in table 1. No attempt was made to remove the air dissolved at room temperature under atmospheric pressure. The hydrogen was commercially pure, cylinder hydrogen and was used without further purification. Mixtures of the naphtha and hydrogen were prepared by first loading the experimental tube with a small sample of the naphtha, calculating its weight from its volume and density, and then injecting into it a measured amount of hydrogen gas. Succeeding mixtures were prepared by adding additional amounts of hydrogen. Details of procedure are given in the papers referred to above.

TABLE 1

Inspection data on the petroleum naphtha used in the hydrogen-naphtha mixtures

COMPOSITION		A.S.T.M. DISTILLATION		
Component	Mole per cent	Volume per cent vaporized	°C.	°F.
Isopentane.....	7.8	Initial	43.3	110
<i>n</i> -Pentane.....	31.3	10	47.8	118
Cyclopentane.....	4.8	20	48.9	120
Neohexane.....	1.7	30	50.0	122
2,3-Dimethylbutane.....	1.7	40	51.1	124
2-Methylpentane.....	16.8	50	53.3	128
3-Methylpentane.....	18.4	60	55.0	131
<i>n</i> -Hexane.....	16.8	70	56.7	134
Residue.....	0.7	80	59.4	139
		90	62.2	144
		Maximum	67.2	153

Density at 15.55°C. (60°F.) = 0.6597 g. per cubic centimeter.

II. RESULTS

Three mixtures containing, respectively, 7.78, 15.22, and 30.21 cc. of hydrogen (at S.T.P.) per gram of naphtha, were prepared and their border curves were determined from about 100°C. upwards through the critical region. The experimental data are given in table 2 and shown graphically by the curves in figure 1. Unfortunately, the critical points were not determined with any great accuracy. However, their approximate locations on the curves are indicated. As will be noted, a minimum point occurs in the boiling-point section of the border curve of two of the mixtures, being most prominent in the mixture of lowest hydrogen concentration.

The relation between the total pressure and the solubility of the gas at constant temperature for several temperatures was determined graphically

from figure 1 and is shown by the curves in figure 2. At the lower temperatures the amount of hydrogen dissolved is directly proportional to the pressure, but as the temperature approaches the critical temperature of the naphtha (about 220°C.), a slight deviation from the linear relation occurs. These constant-temperature lines intersect each other and, when extrapolated to zero amount of hydrogen, cut the pressure axis at what corresponds to the initial vapor pressure of the air-saturated naphtha.

TABLE 2
Boiling points and dew points of mixtures of hydrogen and petroleum naphtha

COMPOSITION: CC. OF H ₂ (AT S.T.P.) PER GRAM OF NAPHTHA	TEMPERATURE	PRESSURE	
		Boiling point	Dew point
	°C.	atmospheres	atmospheres
7.78	55.2	34.8	
	93.3	30.6	
	130.6	29.4	
	148.9	30.3	
	165.6	31.7	14.1
	204.4	37.8	27.4
	216.5	39.5	33.9
15.22	93.3	55.3	
	148.9	47.5	
	165.6	46.8	14.5
	184.0	47.0	20.2
	204.0	47.4	28.8
	217.7	44.4	38.2
30.21	148.9	81.5	
	165.6	76.4	15.6
	183.3	71.7	21.8
	204.4	65.1	32.1
	217.0	53.8	43.1

III. PHASE BEHAVIOR

Unfortunately, the boiling-point curves which contain minimum points (figure 1) were not determined at low temperatures, but their shape may be deduced qualitatively from a knowledge of the solubility of slightly soluble gases in liquids at low temperature. Kuenen (4), reasoning from the known behavior of mixtures, has predicted that the solubility of a slightly soluble gas in a liquid passes through a minimum value at a temperature relatively far removed from the critical point of the liquid. This prediction has been amply confirmed in the investigations of Wiebe and Gaddy (7, 8) on the solubility of hydrogen and helium in water, and of

Wiebe, Gaddy, and Heins (10) and Saddington and Krase (6) on the solubility of nitrogen in water. In view of this property, the solubility of hydrogen in naphtha may be expected to pass through a minimum at some low temperature. As a consequence, the pressure along the boiling-point curve, beginning at the minimum point, will rise at first as the temperature is decreased, pass through a maximum at the temperature of minimum solubility, and finally decrease with decreasing temperature. The com-

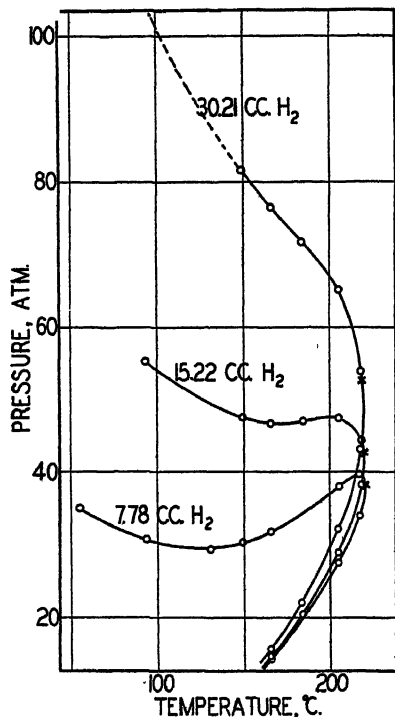


FIG. 1

FIG. 1. Border curves of hydrogen-naphtha mixtures. Composition in cubic centimeters of hydrogen (at S.T.P.) per gram of naphtha. X, critical point.

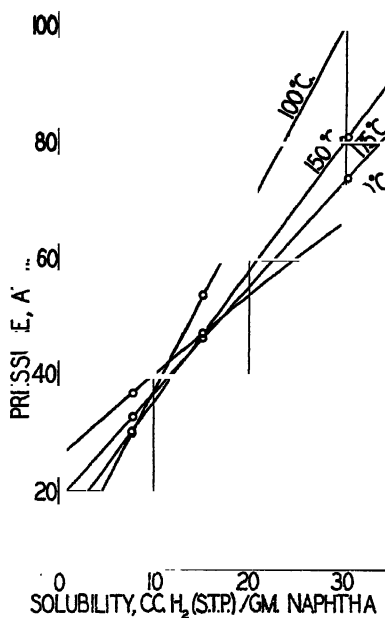


FIG. 2

FIG. 2. Effect of pressure on the solubility of hydrogen in naphtha

plete border curve, then, must look something like that shown in figure 3. The curve *KJHAC* is the boiling-point curve which meets the dew-point curve, *LBC*, in the critical point, *C*. The dotted curves represent curves of constant relative volumes of the liquid and vapor phases in equilibrium, expressed as volume per cent of liquid. In the case of the hydrogen-naphtha mixtures these curves might be expected to be spaced as indicated by the numbers, owing to the low solubility of hydrogen.

The phase behavior when the mixture is heated along the constant pres-

sure line, $P'P''$, brings to light a certain interesting property. At low temperatures no change is observed until D is reached, when a bubble of vapor appears. Between D and E some of the dotted lines are cut twice, indicating that the liquid at first begins to vaporize in the normal manner with the volume of the vapor phase increasing; but as the temperature increases the vaporization comes to a halt, and condensation of the vapor begins and proceeds until it is complete at E . Continuing the heating, no change is observed until F is reached, when vaporization again begins and proceeds in a normal manner until the liquid has been completely vaporized

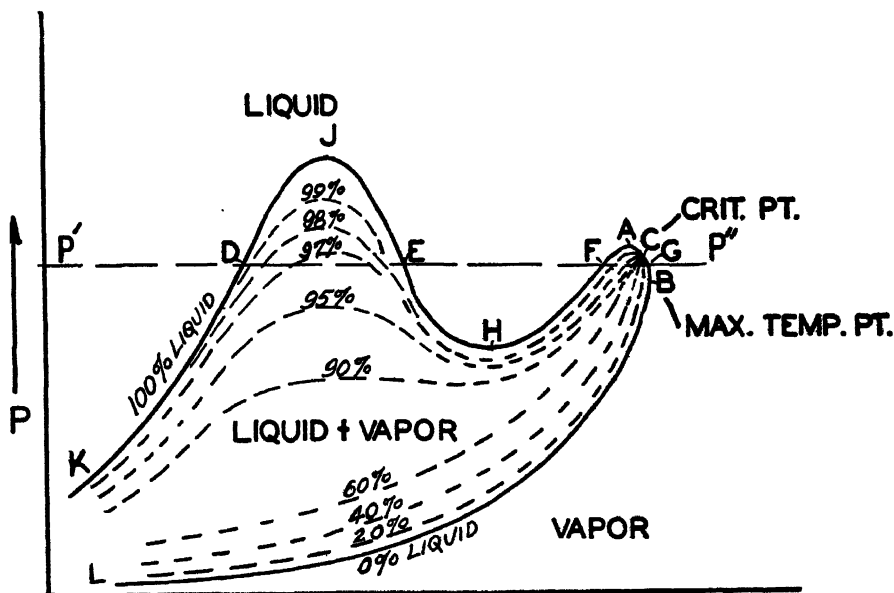


FIG. 3. $P-T$ diagram illustrating the complete border curve of a mixture having a minimum point in its boiling-point curve.

at G . Over part of the path between D and E the phase behavior is the reverse of what usually occurs when a mixture is heated isobarically below its critical pressure. However, it is similar in all respects to what occurs above the critical pressure when the critical point, C , lies to the right of the point of maximum pressure, A . Likewise, it is similar to that described by Kuenen (5) when a mixture the critical point, C , of which lies below the point of maximum temperature, B , is compressed (or expanded) isothermally at a temperature between C and B . Kuenen called this isothermal process "retrograde condensation of the second kind" but did not give a name to the corresponding isobaric process. It would seem logical, there-

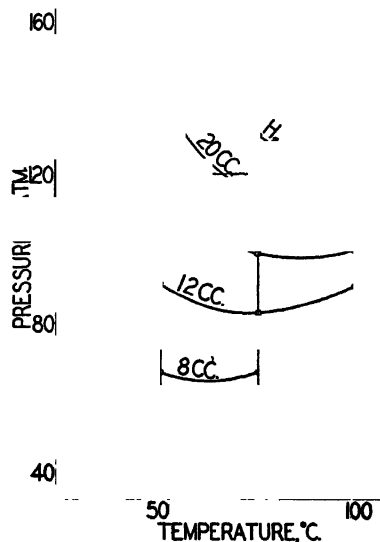


FIG. 4. Boiling-point curves of hydrogen-ammonia mixtures. Composition in cubic centimeters of hydrogen (at S.T.P.) per gram of ammonia. Data by Wiebe and Tremearne (J. Am. Chem. Soc. 56, 2357 (1934)).

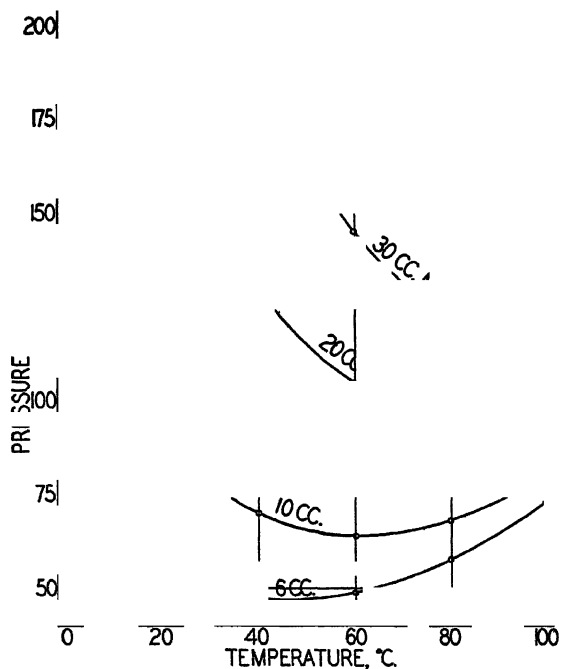


FIG. 5. Boiling-point curves of nitrogen-ammonia mixtures. Composition in cubic centimeters of nitrogen (at S.T.P.) per gram of ammonia. Data by Wiebe and Gaddy (J. Am. Chem. Soc. 59, 1984 (1937)).

fore, to call it "isobaric retrograde condensation of the second kind" or, according to the nomenclature proposed by Katz and Kurata (1), "isobaric retrograde condensation between boiling points."

It will be noted from figure 3 that there are two *P-T* regions where isobaric retrograde condensation of the second kind occurs. One is associated with the critical region of the mixture and over the pressure range from *C* to *A*; the other, with the temperature region immediately below the point of minimum pressure, *H*, and between the maximum pressure, *J*, and a pressure somewhat below *H*.

V. OTHER MIXTURES WITH SIMILAR PROPERTIES

So far as can be determined from the literature, the existence of a minimum in the boiling-point curve has not been pointed out before. However, from the data of Wiebe and Tremearne (11) on the solubility of hydrogen in liquid ammonia and of Wiebe and Gaddy (9) on the solubility of nitrogen in liquid ammonia, boiling-point curves can be derived which show a minimum point. These curves are shown in figures 4 and 5. As in the case of hydrogen-naphtha mixtures, the minimum occurs only over a limited range of concentration from zero concentration of the gas. The temperature range of the data is too narrow to permit the derivation of the complete curves but, in general, they would be expected to be similar to that shown in figure 3.

It would seem that the phase behavior of hydrogen-naphtha, hydrogen-ammonia, and nitrogen-ammonia mixtures might be a characteristic property of a general class of mixtures composed of a liquid and a slightly soluble gas. The investigation of the solubility of such gases as hydrogen, helium, nitrogen, oxygen, and others, in a number of liquids over a wide range of temperature and pressure should prove of considerable interest in testing this generalization.

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PRESSURE AND RATE PROCESSES¹

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When kT times the natural logarithm of the specific reaction rate constant is plotted as ordinate against the pressure as abscissa the slope of the line gives the total difference in volume between the reactant molecules and the activated complex. Here k is the Boltzmann constant and T is the absolute temperature. More precise rules are formulated for predicting the volume increments, for various types of reaction, than those given in earlier papers by Evans and Polanyi or in previous papers from this laboratory. Simple theoretical calculations of the effect of pressure are compared with experimental values for the available unimolecular and bimolecular reactions as well as for viscous flow. The agreement is satisfactory, and the study of pressure effects is seen to lead to the same sort of insight into reaction mechanism that is provided by a study of the temperature effect.

If one knows the volume change in going from the normal to the activated state, the effect of pressure on reaction rates can be predicted in the same way as for equilibria. Formulae for this volume change which depend only on the structure of the reactants are given for unimolecular and bimolecular reactions and for viscous flow and the predicted results are seen to agree well with experiment. When the effect of pressure and temperature on the reaction rates and on the properties of the reactants are known from experiment, it is possible to calculate all the thermodynamic properties of the activated complex. Such calculated results are given for a variety of reactions.

The theory of absolute reaction rates reduces the expression for the rate constant to three factors,—a frequency, an equilibrium constant, and a transmission coefficient

$$k' = \frac{kT}{h} K^\ddagger \quad (1)$$

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where K^\ddagger measures the concentration of activated complex in equilibrium with unit concentration of reactants. In effect, the value of the rate constant gives the value of ΔF^\ddagger , the standard free-energy change for the formation of the activated complex. Measurements of k' through both a temperature and a pressure range, together with data on the compressibilities of the reacting materials, give knowledge of the effect of temperature, of pressure, and of volume on ΔF^\ddagger , and lead directly to six partial derivatives (and implicitly to many others), so that, if the thermodynamic properties of the ground state of the reacting system are known, all the thermodynamic properties of the activated complex may be obtained. The effect of pressure on reaction rates, from the thermodynamic point of view, has been discussed by Evans and Polanyi (7) and by Stearn and Eyring (17).

In this paper are presented the results of a study of rate processes which have been investigated at high pressures. Such processes include viscous flow. The viscosity and compressibility data are those of Bridgman (and are to be found, for the most part, in reference 1). Other thermodynamic data were obtained from *International Critical Tables* and the Landolt-Börnstein *Tabellen*.

In table 1 are given the results of a somewhat detailed study of five processes: the viscous flow of three typical substances—the metal mercury, the hydrogen-bonded liquid glycerol, and ether—the bimolecular reaction between pyridine and ethyl iodide (11), and the monomolecular decomposition of phenylbenzylmethylallylammonium bromide (20).

For fluidities we use a modified form of the equation developed by Eyring (8).

$$\varphi = \frac{\lambda^2 \lambda_2 \lambda_3}{\lambda_1 h} e^{-\frac{\Delta F^\ddagger}{RT}} \quad (2)$$

where we take

$$\frac{\lambda^2 \lambda_2 \lambda_3}{\lambda_1 h} = \frac{V}{N}$$

V being the molal volume and N Avogadro's number.

Thus we have for fluidities

$$\Delta F^\ddagger = RT \ln \frac{V}{\varphi N h} \quad (3)$$

and for chemical reactions

$$\Delta F^\ddagger = RT \ln \frac{kT}{k' h} \quad (4)$$

we use the Lewis and Randall conventions. For values of k' , in place of the unimolecular constant frequently reported for "first-order" bimolecular reactions such as hydrolyses, we use the bimolecular constant. The following are the particular relationships used in calculating the values of the various thermodynamic quantities in table 1:

$$\left(\frac{\partial \Delta F^\ddagger}{\partial P}\right)_T = \Delta V^\ddagger \quad (5)$$

$$\left(\frac{\partial \Delta F^\ddagger}{\partial T}\right)_P = -\Delta S^\ddagger \quad (6)$$

$$\left(\frac{\partial S}{\partial P}\right)_T = \alpha V \quad (7)$$

$$\left(\frac{\partial \Delta F^\ddagger}{\partial V}\right)_T = \frac{1}{\beta_n} - \frac{1}{\beta^\ddagger} \quad (8)$$

$$\left(\frac{\partial \Delta F^\ddagger}{\partial V}\right)_P = \frac{S_n}{\alpha_n V_n} - \frac{S^\ddagger}{\alpha^\ddagger V^\ddagger} \quad (9)$$

$$\left(\frac{\partial \Delta F^\ddagger}{\partial T}\right)_P = \Delta C_P^\ddagger + P(\alpha_n V_n - \alpha^\ddagger V^\ddagger) \quad (10)$$

For calculating the properties of the activated complex in the bimolecular reaction between pyridine and ethyl iodide we have made the following approximations: (1) The partial molal volumes of the two reactants in solution are the same as in the pure liquid state, as are also their coefficients of compressibility and of thermal expansion. (2) Since the molal volumes of pyridine and of ethyl iodide are nearly equal, we use average values of α and β for the normal state. (Data for pyridine are lacking and we assume it to behave under pressure as does phenyl chloride.)

In table 2 we give a more limited number of thermodynamic quantities for a larger number of reactions. For monomolecular reactions, including the process of viscous flow, there is an increase in volume on activation, so that increased pressure decreases the rate of the process. Fawcett and Gibson (9) have studied in a qualitative way the effect of pressures up to 3000 atmospheres on the rates of about fifty organic reactions. In all cases where the reaction proceeded at 1 atmosphere, the rate was increased by increased pressure. These reactions were all at least bimolecular. For the bimolecular reactions cited in tables 1 and 2 (as well as table 3), formation of the activated complex involves a decrease in volume.

For ordinary chemical reactions the volume change on activation at low pressures can be quite accurately calculated, when electrostriction changes

TABLE 1
Results of a detailed study of five processes

P	φ_{∞}°	V	ΔF^{\ddagger}	ΔV^{\ddagger}	ΔS^{\ddagger}	ΔE^{\ddagger}	$\alpha_n \times 10^4$	$\alpha^{\ddagger} \times 10^4$	$\rho_n \times 10^5$	$\beta^{\ddagger} \times 10^5$	ΔC_p^{\ddagger}	ΔH^{\ddagger}
Viscous flow of glycerol												
1	0.263	73.46	6,718	15.0	19.6	12,720	5.3	4.5	2.4	2.0	0	12,720
500	0.193	72.64	6,896	14.2	19.9	12,755	4.7	4.0	2.2	1.82	0.24	12,926
1,000	0.145	71.90	7,061	13.4	20.1	12,826	4.4	3.7	1.96	1.64	0.71	13,151
2,000	0.084	70.61	7,380	12.7	21.1	13,158	4.15	3.5	1.63	1.39	1.29	13,773
4,000	0.0305	68.58	7,970	11.8	21.4	13,310	3.7	3.1	1.34	1.16	4.57	14,454
6,000	0.0119	66.93	8,523	11.2	25.7	14,682	3.3	2.75	1.14	0.99	10.09	16,310
8,000	0.00478	65.50	9,059	11.0	28.3	15,501	3.6	3.0	1.00	0.86	10.04	17,633
10,000	0.00194	64.26	9,590	10.9	30.6	16,219	3.9	3.1	0.88	0.75	13.3	18,860
Viscous flow of ether												
1	471.6	105.5	2,427	20.	-5.0	912	16.9	14.	16.	14.1	0	912
500	304.	98.34	2,650	14.7	-4.55	1,094	11.0	9.5	10.8	9.6	0.3	1,271
1,000	223.6	94.19	2,782	10.8	-4.35	1,204	8.55	7.53	6.8	6.1	0.4	1,464
2,000	144.	89.08	3,039	8.8	-4.05	1,384	5.73	5.1	4.8	4.4	0.1	1,812
4,000	76.1	83.01	3,381	6.8	-4.7	1,486	5.0	4.6	2.8	2.5	0.3	2,148
6,000	42.7	79.31	3,700	6.2	-3.9	1,612	4.42	4.1	1.94	1.8	1.7	2,518
8,000	25.85	76.57	3,984	5.7	-3.6	1,786	3.73	3.42	1.68	1.57	0.6	2,893
10,000	16.0	74.49	4,254	5.5	-3.6	1,830	3.44	3.20	1.48	1.38	3.3	3,163
12,000	10.08	72.64	4,517	5.3	-2.33	2,270	3.26	3.04	1.3	1.21	7.1	3,811
Viscous flow of mercury												
1	65.96	14.84	2,430	0.50	-6.22	545	1.78	2.0	0.40	0.38	0	545
2,000	63.09	14.72	2,454	0.48	-6.24	540	1.67	1.98	0.37	0.36	-0.27	563
4,000	60.13	14.61	2,476	0.50	-6.31	515	1.60	1.86	0.35	0.337	-0.48	564
6,000	57.40	14.51	2,503	0.51	-6.27	529	1.54	1.76	0.325	0.316	-0.68	603
8,000	54.88	14.39	2,526	0.50	-6.34	508	(1.5)	1.4	0.30	0.29		605
10,000	52.30	14.34	2,551	0.52	-6.42	480	(1.76)	1.68	0.27	0.25	-1.4	606
12,000	49.83	14.26	2,576	0.52	-6.31	513	(1.43)	1.61	0.24	0.23	-1.63	664

Pyridine and ethyl iodide in acetone solution, $t_1 = 80^\circ\text{C}$. (reference 11)

P	$k' \times 10^4$	V	ΔF^\ddagger	ΔV^\ddagger	ΔS^\ddagger	ΔE^\ddagger	$\alpha_n \times 10^4$	$\alpha^\ddagger \times 10^4$	$\rho_n \times 10^6$	$\beta^\ddagger \times 10^6$	ΔC_P^\ddagger	ΔH^\ddagger
atm.												
1	0.143	162.6	24,440	-20.	-34.4	14,030	10.4	11.6	7.2	8.0	0	14,030
3,000	1.033	140.6	23,250	-12.7	-28.4	15,568	5.6	4.9	2.5	2.7	7	14,645
5,000	2.13	133.9	22,816	-8.55	-24.4	16,447	4.3	3.5	1.9	2.0	8.6	15,423
8,500	6.5	126.9	22,145	-7.4	-21.5	17,113	4.3	3.5	1.2	1.3	4.7	15,630

Decomposition of phenylbenzylallylmethylammonium bromide in chloroform (20)

P	$k_{25}^\circ \times 10^5$	$k_{45}^\circ \times 10^4$	ΔF_{25}^\ddagger	ΔF_{45}^\ddagger	ΔV_{25}^\ddagger	ΔV_{45}^\ddagger	ΔS^\ddagger	ΔE_{25}^\ddagger	ΔE_{45}^\ddagger	ΔA_{25}^\ddagger	ΔA_{45}^\ddagger	ΔC_P^\ddagger	ΔH^\ddagger
atm.													
1	1.0	1.98	24,237	24,018	3.4	2.25	11.	27,515	27,515	24,237	24,018	0	27,515
3,000	0.66	1.53	24,483	24,181			15.1	28,736	28,819	24,236	24,017	4.15	28,983

TABLE 2
Thermodynamic quantities for certain reactions

REACTION	P	ΔF_1^\ddagger	ΔF_2^\ddagger	ΔH^\ddagger	ΔS^\ddagger	ΔV_1^\ddagger	ΔV_2^\ddagger
	atm.						
Viscous flow: $t_1 = 30^\circ\text{C}.$; $t_2 = 75^\circ\text{C}.$							
$\text{H}_2\text{O} \dots\dots\dots$	1	2,166	1,988	3,378	4.0	0.16	0.83
	4,000	2,272	2,116	3,332	3.5		
	10,000	2,503	2,399	3,200	2.3	1.4	1.3
$\text{CH}_3\text{OH} \dots\dots\dots$	1	2,394	2,442	2,061	-1.1	8.3	9.2
	4,000	2,930	2,988	2,536	-1.3		
	12,000	3,585	3,609	3,434	-0.5	3.2	3.0
$\text{C}_2\text{H}_5\text{OH} \dots\dots\dots$	1	3,013	2,948	3,437	1.4	9.5	11.5
	4,000	3,753	3,802	3,420	-1.1		
	12,000	4,747	4,710	4,989	0.8	4.9	4.3
$n\text{-C}_3\text{H}_7\text{OH} \dots\dots\dots$	1	3,504	3,419	4,080	1.9	15.3	17.4
	4,000	4,565	4,504	4,958	1.3		
	12,000	5,993	5,791	7,357	4.5	6.8	6.0
$i\text{-C}_3\text{H}_7\text{OH} \dots\dots\dots$	1	3,512	3,281	5,057	5.1	19.6	22.0
	4,000	4,770	4,605	5,891	3.7		
	12,000	6,542	6,108	9,451	9.6	9.3	7.3
$n\text{-C}_4\text{H}_9\text{OH} \dots\dots\dots$	1	3,766	3,632	4,675	3.0	18.4	16.5
	4,000	4,965	4,723	6,601	5.4		
	6,000	5,435	5,297	6,374	3.1	9.7	11.8
$i\text{-C}_4\text{H}_9\text{OH} \dots\dots\dots$	1	3,970	3,650	5,738	6.0	21.7	25.1
	4,000	5,477	5,167	7,568	6.9		
	12,000	7,756	7,244	11,210	11.4	11.6	12.4
$n\text{-Pentane} \dots\dots\dots$	1	2,514	2,642	1,666	-2.8	14.8	18.2
	4,000	3,531	3,797	1,743	-5.9		
	10,000	4,529	4,730	3,165	-4.5	7.0	6.0
Isopentane $\dots\dots\dots$	1	2,458	2,593	1,549	-3.0	19.2	22.4
	4,000	3,544	3,799	1,817	-5.7		
	10,000	4,578	4,777	3,245	-4.4	7.0	6.0
$n\text{-Hexane} \dots\dots\dots$	1	2,764	2,910	1,794	-2.2	16.0	20.3
	4,000	3,903	4,154	2,206	-5.6		
	8,000	4,684	4,890	3,290	-4.6	7.7	7.4
$n\text{-Octane} \dots\dots\dots$	1	3,190	3,399	1,796	-4.6	19.9	21.9
	4,000	4,583	4,756	3,432	-3.8		
	6,000	5,111	5,231	4,293	-2.7	4.7	9.8

TABLE 2—Continued

REACTION	P	ΔF_1^\ddagger	ΔF_2^\ddagger	ΔH^\ddagger	ΔS^\ddagger	ΔV_1^\ddagger	ΔV_2^\ddagger
	atm.						
Ethyl bromide.....	1	2,559	2,688	1,832	-2.4	11.4	14.6
	4,000	3,315	3,566	1,618	-5.6		
	12,000	4,302	4,509	2,908	-4.6	4.8	4.4
Ethyl iodide.....	1	2,833	3,031	1,500	-4.4	11.0	12.3
	4,000	3,631	3,882	1,934	-5.6		
	12,000	4,793	4,959	3,672	-3.7	5.9	5.3
<i>n</i> -Butyl bromide.....	1	3,006	3,221	1,552	-4.8	14.3	15.5
	4,000	4,030	4,241	2,606	-4.7		
	12,000	5,620	5,622	5,620	0	8.4	7.0
Acetone.....	1	2,397	2,632	821	-5.2	14.7	12.5
	4,000	3,116	3,339	1,601	-5.0		
	10,000	3,818	3,980	2,727	-3.6	4.6	4.0
Carbon disulfide.....	1	2,403	2,602	1,070	-4.4	8.3	9.7
	4,000	3,000	3,241	1,364	-5.4		
	12,000	3,865	4,061	2,532	-4.4	4.8	4.1
Phenyl chloride.....	1	3,065	3,254	1,792	-4.2	13.8	14.0
	1,000	3,381	3,587	1,987	-4.6		
	2,000	3,673	3,863	2,400	-4.2	12.1	11.4
Phenyl bromide.....	1	3,355	3,564	1,961	-4.6	14.4	14.7
	1,000	3,686	3,905	2,201	-4.9		
	2,000	3,975	4,171	2,642	-4.4	11.9	11.0
Pyridine + cetyl bromide: t_1 = 40°C.; t_2 = 60°C. (10)	1	26,655	26,974	21,647	-16.0	-19.4	-12.1
	1,000	26,185	26,680	18,423	-24.8		
	2,000	25,690	26,354	15,298	-33.2		
	3,000	25,349	26,007	15,051	-32.9	-14.1	-14.3
Acetic anhydride + ethyl alcohol: t_1 = 20°C.; t_2 = 40°C.	1	24,177	24,687	16,195	-25.5	-15.3	-12.7
	1,000	23,807	24,380	14,840	-28.65		
	2,000	23,379	23,950	14,443	-28.55		
	3,000	23,015	23,589*	14,032	-28.7	-15.0	-14.8
In alcohol solution (11, 20)	1	23,879	24,498	14,192	-30.95	-10.0	-11.4
	3,000	23,151	23,672	14,997	-26.05		
	5,000		23,269				
	8,500		22,137				

* Extrapolated from 35°C.

TABLE 2—Concluded

REACTION		P	$\Delta F^\ddagger_{t_1}$	$\Delta F^\ddagger_{t_2}$	ΔH^\ddagger	ΔS^\ddagger	$\Delta V^\ddagger_{t_1}$	$\Delta V^\ddagger_{t_2}$
		atm.						
Ethyl ether + ethyl iodide in alcohol (11, 20): $t_1 = 15^\circ\text{C.}; t_2 = 30^\circ\text{C.}$		1	22,631	22,779	19,791	-9.9		
		3,000	22,366	22,501	19,774	-9.0	-3.6	-3.8
		5,000	22,234	22,330	20,391	-6.4	-2.7	-3.5
		8,500	22,223	22,231	22,070	-0.53		-1.2
		12,000	22,205	22,149	22,317	3.73		-1.0
Alkaline hydrolysis of sodium monochloroacetate (20): $t_1 = 60^\circ\text{C.}; t_2 = 80^\circ\text{C.}$		1	25,875	26,143	21,547	-13.4	-5.7	-6.1
		3,000	25,463	25,698	21,550	-11.8	-4.6	-3.7
		5,000	25,241				-4.9	
		7,600	24,932	25,191	20,620	-13.0	-3.3	
		8,500	24,860				-2.4	
$2\text{H}^+ + \text{Br}^- + \text{BrO}_3 = \text{HBrO} + \text{HBrO}_2$ (13): $t_1 = 15^\circ\text{C.}; t_2 = 25^\circ\text{C.}$		1	16,509	16,607	13,687	-9.8	2.56	2.81
		500	16,540	16,641	13,631	-10.1	2.97	2.48
		1,000	16,576	16,671	13,840	-9.5	2.31	2.56
		1,500	16,602	16,702	13,722	-10.0		
REACTION	CATALYST	P	$\Delta F^\ddagger_{t_1}$	$\Delta F^\ddagger_{t_2}$	ΔH^\ddagger	ΔS^\ddagger	$\Delta V^\ddagger_{t_1}$	$\Delta V^\ddagger_{t_2}$
Acid inversion of sucrose (2,3,18): $t_1 = 15^\circ\text{C.}; t_2 = 25^\circ\text{C. (a)}, 26^\circ\text{C. (b)}$	0.5 M HCl..	1	18,343	18,088 ^a	25,687	25.5		
		500	18,387	18,134	25,653	25.3	3.6	3.8
	0.5 M H ₂ SO ₄ .	1	18,767	18,550 ^b	24,441	19.7		
		500	18,782	18,570	24,340	19.3	1.24	1.65
	0.5 M Oxalic acid	1	19,530	19,375	23,591	14.1		
		500	19,550	19,386 ^b	23,841	14.9	1.65	0.9
	0.5 M Phosphoric acid	1	19,988	19,816	24,942	17.2		
		500	19,954	19,786 ^a	24,792	16.8	-2.8	-2.5
	0.5 M Acetic acid	1	21,552	21,390	25,786	14.7		
		500	21,492	21,441 ^b	22,817	4.6	-4.85	4.2
	n/16 HCl....	1		20,000 ^a				5.0
		500		20,060				5.3
		1,000		20,124				4.3
		1,500		20,176				
$t_1 = 25^\circ\text{C.}$ $t_2 = 45^\circ\text{C.}$	0.5 M Acetic acid	1	21,875	21,659	25,093	10.8	-0.9	-2.0
		500	21,864	21,635	25,276	11.4	-1.4	-1.2
		1,000	21,847	21,620	25,229	11.4	0	0
		1,500	21,847	21,620	25,229	11.4		

on activation are not important, by the use of two simple rules: (1) For a monomolecular reaction

$$\Delta V^\ddagger = \frac{0.1l^\ddagger}{\sum_i l_i + r_1 + r_2 + 1} V \quad (11)$$

where l_1 is the sum of the bond lengths taken in the direction of decomposition, r_1 and r_2 are the covalent radii of the terminal atoms at either end, V is the molal volume, and l^\ddagger is the length of the bond which breaks and which, in the activated complex, has lengthened by about 10 per cent. The number 1 added to the denominator is twice the distance from the covalent (or electrovalent) "shell" of the outer atom to the kinetic theory shell of the molecule. This number is taken from the fact that a hydrogen atom in water is about 0.8 Å. nearer the oxygen atom to which it is covalently bonded than to the next nearest oxygen atom (16), so that this distance will, in general, be somewhat larger than and not differ much from 1 Å.

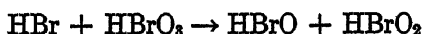
(2) For bimolecular reactions

$$-\Delta V^\ddagger = \frac{1}{\sum_i l_i + \sum_j r_j + 2} \sum V \quad (12)$$

In equation 12 we assume that, from the sum of the molal volumes of the reactants, $\sum V$, we squeeze out the 1 Å. between them when the activated complex is formed. $\sum_i l_i$ is the sum for both molecules of the sum of the interatomic distances between the remote end and the reacting end of each molecule, and the r 's are the covalent (or ionic, if the reacting atom is charged as, for example, oxygen in HO^-) radii of the end atoms and of the reacting atoms of the molecules.

In table 3 we compare measured values of ΔV^\ddagger with values calculated by means of equation 11 or 12.

Two reactions are particularly worthy of comment. Moesveld (13) studied the kinetics of the reaction



in aqueous solution to pressures of 1500 atmospheres. This reaction has been shown by Judson and Walker (12) to be of the fourth order. Ordinarily it might be expected that pressure would accelerate its rate. The reverse effect was found. In this reaction, however, four ions combine to form two molecules of weak electrolyte, so that the formation of the activated complex is accompanied by the liberation of electrically bound solvent to an extent which overbalances, though not greatly, the decrease in volume otherwise to be expected. The net effect amounts to a volume

TABLE 3
Comparison of experimental and calculated values of ΔV^\ddagger

REACTION AND REFERENCE	PRESSURE RANGE	ΔV^\ddagger EXPERIMENTAL	ΔV^\ddagger CALCULATED
	atmospheres	cc.	cc.
Decomposition of methylallylbenzylammonium bromide (20).....	1-3000	3.4	3.5 for breaking methyl bond 2.7 for breaking any other bond
Pyridine + ethyl iodide (11).....	1-3000	-16.3	-15.8
Pyridine + cetyl bromide (10).....	1-1000	-12.5	-12.3
Acetic anhydride + ethyl alcohol (20)..	1-1000 (in alcohol)	-14.0	-13.3 for 1 C ₂ H ₅ -OH per 1 anhydride
	1-3000 (in toluene)	-12.2	
Alkaline hydrolysis of ClCH ₂ COO ⁻ (20)	1-3000	-6.	-8.
Ethoxide + ethyl iodide (20).....	1-3000	-4.	-11.
Alkaline saponification of ethyl acetate (4).....	250-500	-5.5	-11.
Ethyl o-methylcinnamate (14).....	1-500	{ -14.0 (in 31% alcohol) -12.4 (in 42% alcohol)	-12.3
Cinnamyl cinnamate (15).....	1-1500		
Ethyl o-methoxycinnamate (15).....	1-1500	{ -14.5 -15.5 (in 25% alcohol) -12.1 (in 38% CH ₃ OH) -16.8 (in 28% acetone) -16.2 (in 20% C ₂ H ₅ OH)	-12.7
Ethyl benzoate (15)..<	1-1500		
Ethyl o-methoxybenzoate (15).....	1-1500		
Benzyl benzoate (15).	1-1500		
Ethyl anthranilate (15).....	1-1500	-10.4	-11.6
Linalyl acetate (15)..<	1-1500	-16.2	-17.4
Bornyl acetate (15)..<	1-1500	{ -24.5 (in 31% alcohol) -22.1 (in 41% alcohol) -24.5 (in 19% C ₂ H ₅ OH) -20.4 (in 3.5% pyridine) -25.0 (in water)	-20.1
2H ⁺ + Br ⁻ + BrO ₃ ⁻ (13).....	1-500	2.6	
Acid inversion of sucrose (2, 3, 18)..<	1-500	1 to 5 in HCl, H ₂ SO ₄ , or oxalic acid -2 to -5 in phosphoric or acetic acid	

increase of between 2 and 3 cc. (table 2). We, therefore, expect that in this case ΔV of reaction will be in the same direction as ΔV^\ddagger of activation and will be a little larger. The other reaction is the acid inversion of cane sugar. Cohen and de Boer (3; see, also, 2 and 18) have found a small but unequivocal pressure effect on the rate. In this reaction the effect of pressure on the rate depends on the nature of the catalyst. Specifically, for example, pressure increases the rate in the presence of acetic acid but decreases it in the presence of hydrochloric acid. This result indicates that either chloride ion or acetate ion, or both, are built into the activated complex, or their presence would not affect ΔV^\ddagger . But if they are to be built into the activated complex they must release some of their water of hydration, which should be more in the case of chloride ion than of acetate ion. Thus one might expect that ΔV^\ddagger should be more positive for chloride ion than for acetate ion, as in fact it is. In no case is the effect large, and the next volume change results from a balance between loss of volume when sucrose, water, and catalyst combine into activated complex, and a volume increase due to the opening up of the large sucrose molecule on activation. The significance of this second factor may be inferred from the fact that ΔS^\ddagger is in all cases positive.

Polymerization reactions have been studied at high pressures by Tammann and Pape (19) as well as by Conant *et al.* (5, 6). Pressure has a very large accelerating effect on the rate. The necessity of well-controlled catalytic environment renders the data slightly less trustworthy for quantitative interpretation, but the rate of polymerization of isoprene under roughly comparable conditions increases nearly 6000 times at room temperature between pressures of 2000 and 18,000 atmospheres, according to measurements of Conant and Tongberg, and the rates are nearly proportional to the fourth power of the pressure through this entire pressure range. Tammann and Pape, working at higher temperatures and through the pressure range of about 1000 to 3000 atmospheres, found that under these conditions the rates of polymerization of isoprene, styrene, and dimethylbutadiene increase as the second power of the pressure in the first two cases and as the fourth power in the case of dimethylbutadiene. The data of Conant and those of Tammann for isoprene seem quite comparable, since temperature extrapolation of the rate constant at 2000 atmospheres, using the mean of the values of activation given by Conant and Tongberg for room temperature and by Tammann and Pape for 150°C., bring the two values of the rate constant to within a factor of about 2, though the initial constants differed by over 2×10^4 .

It is generally accepted that these polymerization reactions have chain mechanisms. Their rates may be instructively formulated in terms of the factors which effect the starting and the breaking of the chains. For the

rate of reaction of a radical (i.e., the chance that, in unit time, a chain will be broken), we write

$$1/\tau_{\text{ave.}} = C_w k_2 + \sum_i C_b_i k_{1_i} \quad (13)$$

where C_w is the effective concentration of wall molecules (i.e., the number of reactive surface atoms per cubic centimeter of reacting medium) and C_b is the concentration of any other molecular species which inhibits the reaction by means of breaking the chain. k_2 and the k_{1_i} 's are the corresponding rate constants. k_2 will depend on the nature and shape of the wall and the rate of diffusion to the wall; k_1 will depend on the particular reaction and on the diffusion velocity. τ is the life period of the chain. We use an average value since, for such reactions as polymerizations, increase in chain length involves actual increase in the size of the reacting radical, and thus the older the chain the smaller the chance of breaking due to its diffusion (e.g., to the wall).

The measured rate of reaction would then be

$$k' = C_r k_3 \tau_{\text{ave.}} = \frac{C k_4 k_3}{C_w k_2 + \sum_i C_b_i k_{1_i}} \quad (14)$$

where C_r is the concentration of radicals, and k_3 is the rate constant for the growth of a chain. For C_r we write $C k_4$, where C is the concentration of the parent substance and k_4 measures the rate of initiation of chains. If k_1 , k_2 , k_3 , and k_4 are independent of C , the reaction will be of the first order, as is observed. Positive catalysts,—as, for example, peroxide in the polymerization of isoprene,—probably act by increasing k_4 . In general, negative catalysts may act as chain breakers, increasing the denominator of the right-hand side of equation 14, or they may presumably decrease the values of either k_3 or k_4 .

Conant and Tongberg give the activation energy for polymerization of isoprene at room temperature and 2000 atmospheres as about 24,000 calories, whereas the data of Tammann and Pape at 2000 atmospheres indicate an activation energy at 150°C. of about 17,300 calories. From equation 14 it is seen that the measured value of the activation energy is the difference between the sum of those energies characteristic of k_3 and k_4 and some sort of average of the analogous energies characteristic of k_2 and the k_{1_i} 's. It is quite possible that the dominant chain-breaking mechanism may be an entirely different one at high temperatures than at lower temperatures, so that one need not be surprised at large temperature variation of activation energy.

While values of ΔS^\ddagger may not have the same significance for these reactions as for the others treated in this paper, it may be pointed out that,

from data available, they are negative and rather large, ranging from -30 to -50 units.

The effect of pressure on polymerization rates is in a sense slightly easier to analyze than is that of temperature. Naturally, increasing the pressure increases C and the C_b 's and perhaps C_w , but such effects are small compared with the effect on k' itself. The somewhat complicated net pressure effect on the polymerization rate will manifest itself as simple changes in the various specific rate constants on the right-hand side of equation 14.

If we accept the idea of Conant and Peterson as to the function of peroxide decomposition in initiating chains in isoprene polymerization, and assume some analogous mechanism in the general case, then we expect k_4 to be nearly independent of pressure, though it should decrease slightly. This would accord with the findings of Williams, Perrin, and Gibson (20) as to the effect of pressure on the monomolecular decomposition of phenylbenzylallylmethylammonium bromide, the rate of which decreases about 33 per cent on going from 1 atmosphere to 3000 atmospheres. We may, therefore, think of k_4 as nearly independent of pressure or as decreasing as a small fractional power of the pressure.

On the evidence of the effect of pressure on the rates of those bimolecular reactions for which data are available and for which, on the average, k' increases about as the first power of pressure (starting with an initial pressure of at least 500 atmospheres), we expect k_3 to vary about as the first power of the pressure. k_2 and the k_1 's should depend on pressure in much the same way as does fluidity. Now we find that, in the range of about 5000 to 12,000 atmospheres at 30°C ., viscosity varies nearly as the cube of the pressure. This power is an average based on data for the three hydrogen-bonded liquids *n*-propyl alcohol, isobutyl alcohol, and glycerol, and the three more normal liquids *n*-pentane, ethyl bromide, and carbon disulfide. We would then expect that the speed of polymerization at room temperature would increase about as $P^{0.1+0.3}$ or as P^4 , in good agreement with the data of Conant and Tongberg for isoprene polymerization at room temperature. The measurements of Tammann and Pape on the same reaction were made at higher temperatures (*ca.* 150°C .) and lower pressures (1000 to 3000 atmospheres in place of the 2000 to 18,000 atmospheres covered by Conant and Tongberg). In the pressure range 1000 to 3000 atmospheres the viscosities of the six liquids enumerated above vary, on the average, with $P^{0.9}$ at 30°C ., changing to $P^{0.8}$ at 75°C ., and presumably to a somewhat lower power of pressure at 150°C . This is entirely consistent with the finding, by Tammann and Pape, of a rate of isoprene polymerization increasing as P^2 under their experimental conditions.

According to the mechanism pictured above for polymerization reactions, the calculated value of ΔV^\ddagger will be a composite ΔV^\ddagger for diffusion and

ΔV^\ddagger for polymeric addition. Now the density of the isoprene monomer is 0.68, while that of the polymerized material is 0.9. Thus the complete reaction involves a volume decrease of 24.5 cc. per isoprene unit. The formation of the activated complex would involve slightly less. On the basis of equation 11 it would involve a decrease of 0.9 of 24.5, or 22 cc., per isoprene unit. In diffusion or viscous flow the volume increase on activation averages about one-seventh the volume of the diffusing segment. Thus if X be the number of isoprene units in the diffusing segment, then

$$\Delta V_{\text{meas.}}^\ddagger - 22X = \frac{V_c X}{7} \quad (15)$$

V_c is the volume per isoprene unit of the condensed chain. Since there are two double bonds in isoprene, we use 22 cc. as the volume shrinkage for formation of the activated complex between two reacting units in place of twice this value if there were only one point of condensation per isoprene unit. For numerical substitution we should know the value of $\Delta V_{\text{meas.}}^\ddagger$ at low pressures (since relative compressibilities of monomer and polymer are not known over a pressure range). The data of Tammann and Pape give $\Delta V^\ddagger = -66$ cc. for the pressure range 650 to 1140 atmospheres, and $\Delta V^\ddagger = -266$ cc. for the pressure range 1 to 1500 atmospheres. These values of ΔV^\ddagger correspond, respectively, to about four and twenty-two isoprene units as constituting the diffusing segment.² The former value is probably more nearly correct, since the experimental pressure effect on k' at 1 atmosphere is highly questionable.

In the case of styrene, the density of the monomer being 0.902 and that of the polymer "1.043 to 1.056" (19), we take 1.05 as the density of the polymer and obtain a volume decrease in going from polymer to activated complex of 14 cc. per styrene unit. For measured values of ΔV^\ddagger , Tammann's data yield -84 cc. for the pressure range 740 to 1300 atmospheres, and -327 cc. for the pressure range 1 to 1500 atmospheres. These values of ΔV^\ddagger correspond respectively to about four and twenty-one styrene units as constituting the diffusing segment, the former value being probably more nearly true.

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THERMODYNAMIC CALCULATIONS OF PRESSURE EFFECTS IN GASEOUS REACTIONS BY THE EQUATION OF STATE METHOD. A BRIEF REVIEW^{1,2}

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The effect of pressure, temperature, and the composition of the equilibrium mixture on the mass-action function K_p is briefly discussed with special reference to published computations for the Haber equilibrium. Improvement in the accuracy of calculations of K_p lies in two directions: improved equations of state for pure gases and improved rules for combining constants (or groups of constants) for the mixture equation. Recent advances in these directions are mentioned.

The basis underlying thermodynamic calculations of the effect of pressure on the yield of a gas reaction may be explained as follows:

It is a general exact principle that an increase of pressure at constant temperature increases the yield of a reaction if the result of the reaction at constant temperature and pressure would be to decrease the total volume of the system.

If the system is one of ideal gases, the equilibrium constant K_p (in terms of the pressure times mole fraction of products) is constant at constant temperature.

If the gases are not ideal, the function K_p is still convenient, though not constant with varying pressure. In this case there is an equation

$$RT (d \ln K_p / dp)_T = (-\delta V / \delta n)_{T,p} - (-\delta V / \delta n)_{T,p,ideal}$$

the right-hand side of which may be explained as the actual volume decrease minus the decrease as calculated for a mixture of ideal gases, these volume decreases being per mole of some species formed in a supposed infinitesimal shift of equilibrium at constant temperature and pressure.

¹ Presented at the Symposium on Reactions and Equilibria in Chemical Systems under High Pressure, which was held under the auspices of the Division of Physical and Inorganic Chemistry at the One-hundredth Meeting of the American Chemical Society, Detroit, Michigan, September 9-13, 1940.

² Contribution No. 464 from the Research Laboratory of Physical Chemistry of the Massachusetts Institute of Technology.

The species can be any one of the products of the reaction, so long as the chemical equation, to which K_p corresponds, is written for the formation of 1 mole of it.

This equation is rigorously exact, if we note that the derivative on the left is for frozen equilibrium (as is most convenient for calculations) and does not include the small secondary effect of shift of equilibrium on K_p . When integrated, it is equivalent to one already given (2).

The small effect of composition on K_p at constant temperature and pressure cannot be explained in an equally simple manner, since the corresponding equation involves a pressure integration.

The actual volume decreases are not measured. According to the Lewis and Randall fugacity rule, they may be calculated from the actual molal volumes of the various pure species under the assumption that volume changes on mixing the gases at constant temperature and pressure are zero.

Better results are obtained by the use of an equation of state for mixtures, in which the constants for the mixture are calculated from the constants of the pure gases and their mole fractions according to certain combination rules. These rules are somewhat arbitrary but involve no new or arbitrary constants. A summary of these methods has been given in a review (3).

A complete typical application of the method has been made to the Haber equilibrium (4). All available data were smoothed within the experimental error with the use of only two adjustable constants. These may be taken to represent one value of K_p and one value of ΔH . Contrary to an opinion expressed by others, no arbitrary constants were used in the (complete) explanation of the pressure effect (3).

The experiments as well as the calculations are so complete for the Haber equilibrium that some of the results are of general interest in considering the effect of high pressure on gaseous chemical equilibria.

In the first place, the equilibrium yields are already so high at 1000 atmospheres that a beneficial result of increasing the pressure still more would seem to depend principally on an increase of reaction velocity, or on a better balance of engineering factors. At 450°C. and 1000 atmospheres an equilibrium mole per cent of ammonia of 69.4 has been observed (68.6 calculated). This is nearly 82 per cent ammonia by weight.

In considering engineering factors, the effect of pressure on the heat of reaction must not be forgotten. At 500°C. this was calculated to be about 20 per cent for a pressure increase of 1000 atmospheres.

The theoretical effect of a changing hydrogen:nitrogen mole ratio on the value of K_p was found to be small, so long as the ratio is kept at about 3. Thus at 1000 atmospheres no practical benefit on the equilibrium yield can be obtained by using the theoretical optimum ratio instead of the stoichiometrical ratio.

Improvement in the accuracy of calculation of mass-action deviations by the equation of state method lies in two directions: improved equations of state for the pure gases and improved rules for combining constants (or groups of constants) for the mixture equation. Such improvements will doubtless be at the expense of more tedious calculations.

Data on chemical equilibrium are not very helpful in studying possible improvements in the combination rules, as the chemical calculations are not very sensitive to small changes in the rules. What they require most is good v dp integrals for the pure gases, and then approximate values for the terms involving volume changes on mixing gases. This is fortunate for the application to chemical problems, but it means that further progress in the problem waits on more p - v - T data, both for pure gases and for mixtures.

A brief discussion of some important work in this field follows.

Benedict, Webb, and Rubin (1) give an equation of state for hydrocarbons (pure) that represents the compressibility to about twice the critical density and also the changes in curvature of the isometrics at about the critical and twice the critical density. Vapor pressures as well as saturation liquid and gas densities may be calculated with extraordinary accuracy.

According to Professor J. A. Beattie, unpublished computations indicate that square-root combination for A_{om} and c_m , together with Lorentz combination for B_{om} and linear combination for a_m and b_m in the Beattie-Bridgman equation for mixtures, gives somewhat the best representation of the compressibility of hydrocarbon mixtures.

An important recent attempt to apply the equation of state method to gases for which individual equations of state are not yet available is that of Keyes (5). He has derived an approximate reduced equation of state which is sufficient for many purposes. He expresses the second virial coefficients in terms of two sets of numerical coefficients—one for polar, and one for non-polar gases—and the critical temperature and pressure of the substance. He also applied the equations to several thermodynamic calculations with surprisingly good results.

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ISOMERIZATION OF UNSATURATED HYDROCARBONS¹

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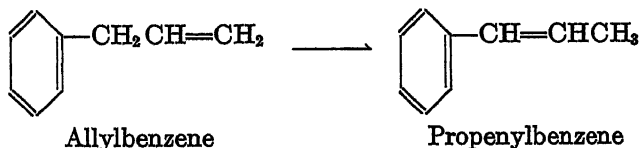
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Of all the isomerizations of hydrocarbons, only those of unsaturated ones allow a clear formulation, and even here there exist certain reactions, occurring under rather abnormal conditions of pressure and temperature, which are far from being describable by the usual valency scheme, e.g., the conversion of butene into isobutene (3, 52, 80). This article deals exclusively with the isomerization reactions of unsaturated hydrocarbons, which may be divided into two groups: (I) isomerization of open-chain hydrocarbons into other open-chain hydrocarbons; (II) isomerization of open-chain hydrocarbons into cyclic hydrocarbons. The reversal of the latter process is rare,—a case in point is the thermal conversion of cyclopropane into propene (6, 68),—and the reactions that we shall discuss under this heading cannot be reversed at all.

I. ISOMERIZATION OF OPEN-CHAIN HYDROCARBONS INTO OTHER OPEN-CHAIN HYDROCARBONS

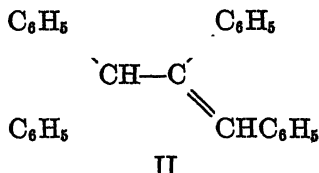
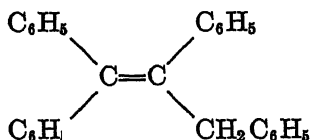
Among these reactions, which proceed through the migration of a hydrogen atom in a triad system ($C=C-C$), the direction of the isomerization is generally determined by the tendency to form a conjugated system. Thus, the alkaline conversion of allylbenzene into propenylbenzene (5) and similar reactions (60a) are obviously provoked by the stability of the phenyl-double bond system in the end product:



A similar case is the isomerization of 1,4-pentadiene into 1,3-pentadiene (57, 87). In view of the olefinic character of the 9,10-double bond in phenanthrene, the isomerization of 9-allylphenanthrene into 9-propenylphenanthrene (11) may also be quoted here. While in these cases the

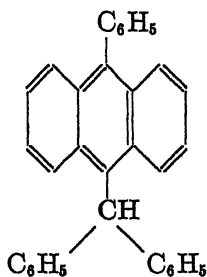
¹ One of the meetings of the Faraday Society ("Hydrocarbon Chemistry," *Trans. Faraday Soc.* **35**, 806 *et seq.* (1939)) dealt with the isomerization of unsaturated hydrocarbons more from the physical point of view. It seemed desirable, therefore, to present a review of the chemical data pertaining to this problem.

reaction is practically non-reversible, all those systems in which both compounds contain conjugated systems are reversible (mobile in both reactions), as in the cases of indene and of 1,3-diphenylpropene, both of which have been studied extensively by Ingold and coworkers (2). Other examples studied are the following: (a) the pair 1,1,2-triphenyl-2-benzylethylene (I) and 1,2-diphenyl-1-benzhydrylethylene (II) (20);

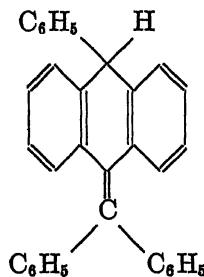


1,1,2-Triphenyl-2-benzylethylene 1,2-Diphenyl-1-benzhydrylethylene

(b) the pair 9-benzhydryl-10-phenylanthracene (III) and 9-benzhydrylidene-10-phenyl-9,10-dihydroanthracene (IV) (7);

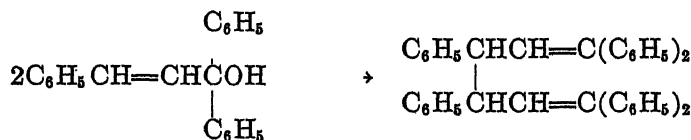


III
9-Benzhydryl-10-phenyl-
anthracene



IV
9-Benzhydrylidene-10-phenyl-
9,10-dihydroanthracene

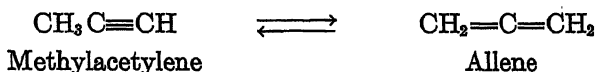
(c) the spontaneous formation of 1,1,3,4,6,6-hexaphenyl-1,5-hexadiene (VI) on the reduction of diphenylstyrylcarbinol (V) (61):



V
Diphenylstyrylcarbinol

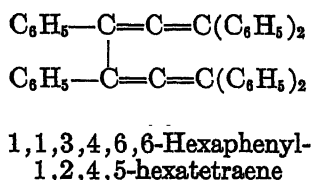
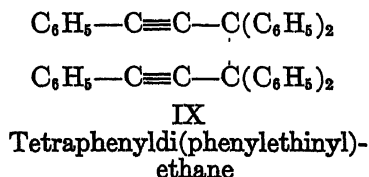
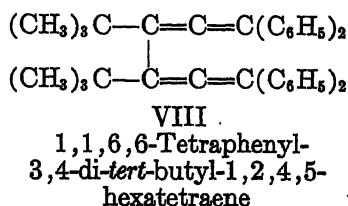
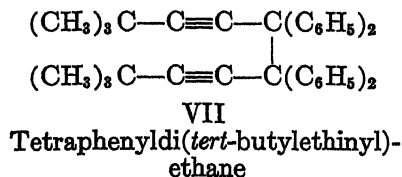
VI
1,1,3,4,6,6-Hexaphenyl-1,5-
hexadiene

The genetic relationship between methylacetylene and allene (4), which also appears in their substitution products (53), should be mentioned here:



In recent years an analogous transformation has been observed by Marvel and coworkers (47, 48, 62, 66, 82, 83, 88) in a series of highly substituted

acetylenic hydrocarbons which rearrange irreversibly into the isomeric allenes: tetraphenyldi(*tert*-butylethynyl)ethane (VII) gives 1,1,6,6-tetraphenyl-3,4-di-*tert*-butyl-1,2,4,5-hexatetraene (VIII) (47); tetraphenyldi(phenylethynyl)ethane (IX) gives 1,1,3,4,6,6-hexaphenyl-1,2,4,5-hexatetraene (X) (66), which had been obtained previously by Moureu, Dufraisse, and Houghton (65):

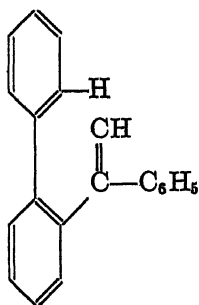


II. ISOMERIZATION OF OPEN-CHAIN HYDROCARBONS INTO CYCLIC HYDROCARBONS

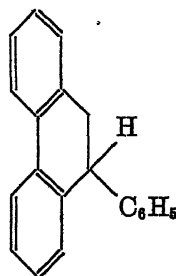
The cases in which the rearrangement of an open-chain hydrocarbon consists in a "cycloisomerization" are much more frequent, various types of reaction being known.

A. Cycloisomerization of a vinylbenzene system

The only two cases of the cycloisomerization of a vinylbenzene system known at present are (a) the isomerization of *as*-phenyl-*o*-biphenylethylene (XI) into 9-phenyl-9,10-dihydrophenanthrene (XII), observed under the influence of phosphorus pentachloride (11, 14),

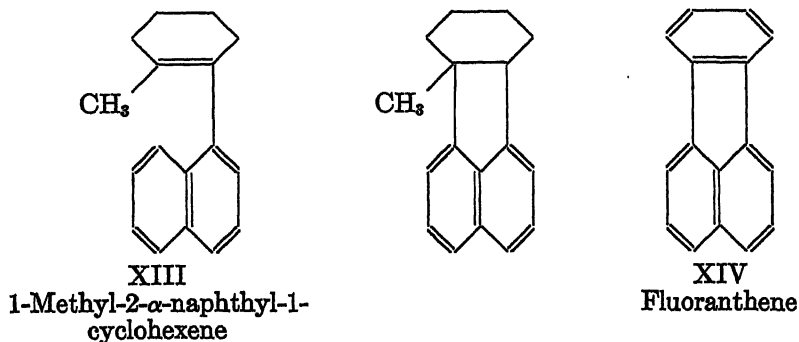


XI
as-Phenyl-*o*-
biphenylethylene



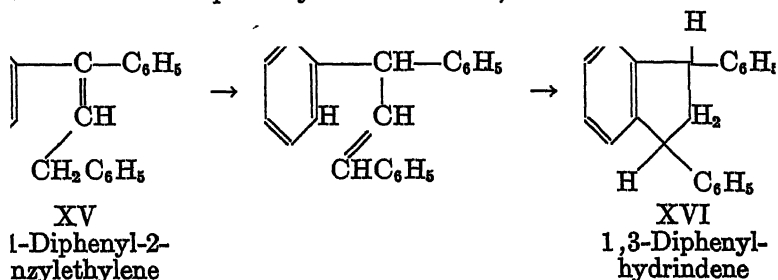
XII
9-Phenyl-9,10-dihydro-
phenanthrene

and (b) the isomerization of 1-methyl-2- α -naphthyl-1-cyclohexene (XIII), which, by cyclization and subsequent dehydrogenation, gives fluoranthene (XIV) (41):

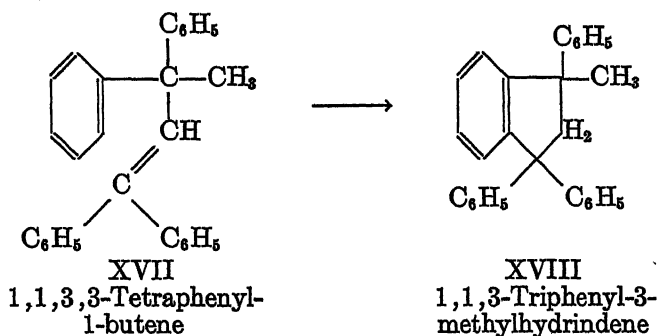


B. Cycloisomerization of an allylbenzene system

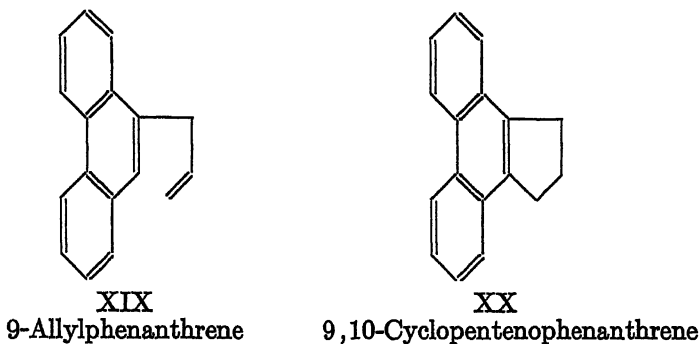
The first cases of this type studied were (a) the conversion, by means of concentrated sulfuric acid, of 1,1-diphenyl-2-benzylethylene (XV) into 1,3-diphenylhydrindene (XVI) (77), a change which obviously involves two isomerization reactions,—namely, the migration of hydrogen in the open chain and subsequent cycloisomerization,—



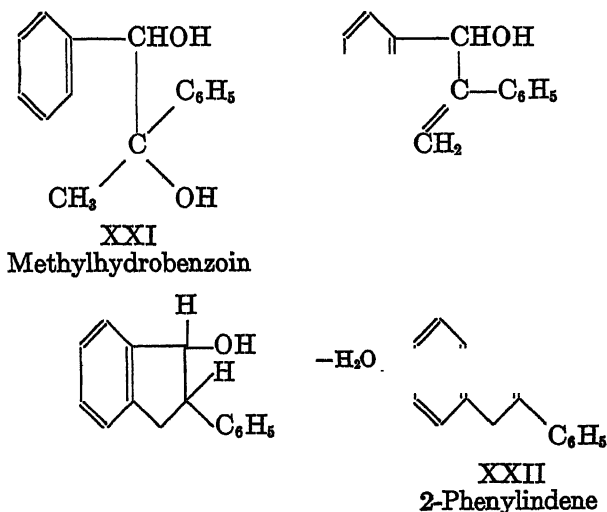
and (b) the isomerization of the unsaturated isomer of *as*-diphenylethylene (1,1,3,3-tetraphenyl-1-butene) (XVII) into the saturated isomer, 1,1,3-triphenyl-3-methylhydrindene (XVIII) (18):



Other cases are the isomerization of the unsaturated dimer of anethole into 6-methoxy-1-(*p*-methoxyphenyl)-2-methyl-3-ethylhydrindene (2a), the cycloisomerization of α -benzylstilbene (19), the cycloisomerization of 2,2-diphenyl-4-methyl-3-pentene (17), and the cycloisomerization of 9-allylphenanthrene (XIX), which is transformed into 9,10-cyclopentenophenanthrene (XX) (12a):

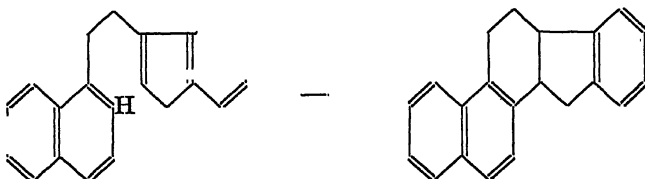


It has been further discovered (24) that a whole group of compounds, the alkylhydrobenzoin, are converted into indene hydrocarbons by dehydrating agents according to a similar scheme. An example is the conversion of methylhydrobenzoin (XXI) into 2-phenylindene (XXII).

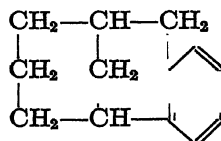


This isomerization has subsequently found much use in the synthesis of compounds related to sterols (33, 37, 38; 27, 28a; 59; 12, 13, 51, 78),—the sterol molecule contains a hydrindene system,—but could not be

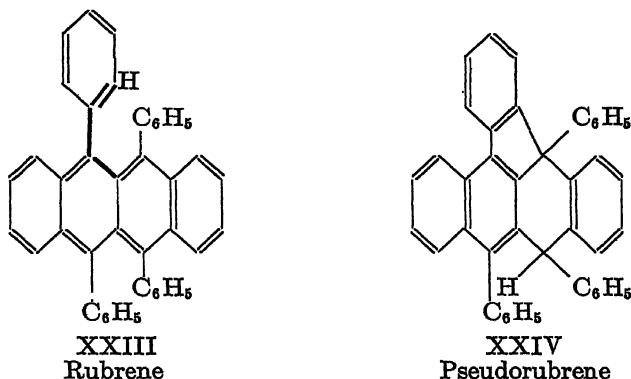
extended by Cook and coworkers (1, 35, 37, 40) to include the building up of fluorene derivatives, e.g.,



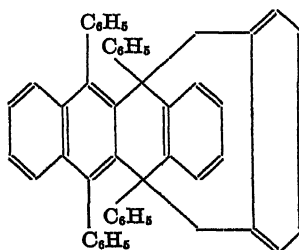
in general. In the case of benzylcyclohexene, Cook and Hewett (39) have shown that no fluorene derivative is obtained, but, rather unexpectedly, a substance of the following structure:



A curious isomerization which converts the red rubrene (9,10,11,12-tetraphenylnaphthacene, XXIII) into the colorless so-called pseudorubrene² (XXIV) (45, 46, 64) may well be explained by a similar cycloisomerization.



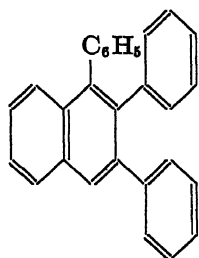
² Moureu and his coworkers, however, prefer another formula for pseudorubrene, in which a benzene ring with two ortho carbon atoms forms a bridge between the positions 11 and 12:



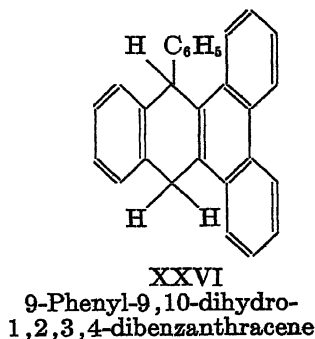
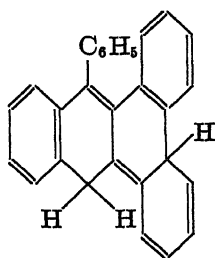
This formula seems to be in contradiction with the known stability of ring valencies toward deflection.

The molecule contains the system of an allylbenzene (marked in heavy type), which is isomerized as usual except that the hydrogen atom does not remain at the central atom of the allyl system; it subsequently undergoes an allylic rearrangement and the hydrogen appears therefore in position 12. An analogous observation has been made in the case of 1,4,9,10-tetra-phenylanthracene (90). It may be added that *formally* the isomerization of *as*-phenyl-*o*-biphenylethylene, mentioned above, may also be regarded as a case of allylbenzene isomerization, the single bond of the allylic system being located in the benzene nucleus.

A similar, but still more complicated, case of cycloisomerization is the conversion of 1,2,3-triphenylnaphthalene (XXV) into 9-phenyl-9,10-dihydro-1,2,3,4-dibenzanthracene (XXVI), which, according to Schlenk and Bergmann (76; see 10, 23), takes place under the influence of metallic lithium. The following scheme assumes an intermediate transfer of hydrogen along a system of conjugated double bonds:

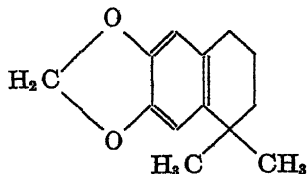
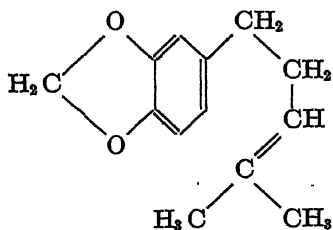


XXV
1,2,3-Triphenyl-
naphthalene

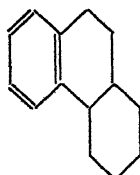
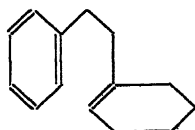


C. Cycloisomerization of a butenylbenzene system

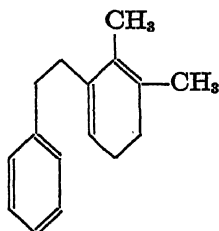
Bogert and coworkers (25, 26, 28, 67, 70, 71) were the first to synthesize hydrogenated naphthalenes and phenanthrenes from butenyl-substituted benzenes (or the corresponding alcohols): for example,



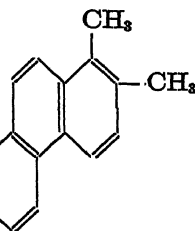
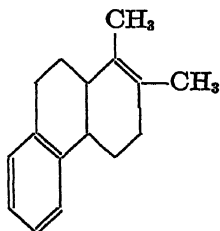
and



Subsequently, this method was also used by other investigators for various syntheses: e.g., (a) for the preparation of 1,2,5,6-dibenzphenanthrene from 4-(β -phenylethyl)-1,2-dihydrophenanthrene (8), (b) for the preparation of 1-methyl-6-isopropylphenanthrene (79a), (c) for the synthesis of triphenylene derivatives (e.g., triphenylene itself from 1-phenyl-2-cyclohexenylcyclohexanol (71a)), and (d) for the synthesis of dodecahydro-1,2-benzanthracene from 2-(β -phenylethyl)octalin (42; for further references see 70). The following case, in which a more unsaturated cyclization product is formed, may also be cited (21): the condensation product (XXVII) resulting from the reaction of 2,3-dimethyl-2-cyclohexen-1-one and β -phenylethylmagnesium chloride is converted, through cyclization and subsequent dehydrogenation, into 1,2-dimethylphenanthrene (XXVIII).



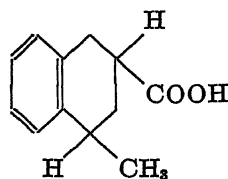
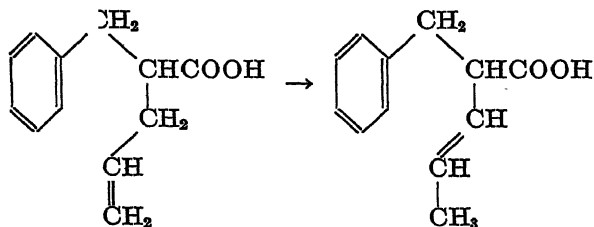
XXVII

XXVIII
1,2-Dimethyl-
phenanthrene

In all these cases (see page 539) the usefulness of the reaction is sometimes decreased by the formation of spirans. These spirans can easily be detected, as they remain unchanged under suitable dehydrogenation conditions; their structure can be elucidated by oxidation experiments. It has been reported that methyl groups in the γ -position of the butenyl side chain favor the "normal" ring closure in this series (69, 70).

With regard to the cyclization proper, the Bogert synthesis is identical with the method reported by Bardhan and Sengupta (2b) a short time before; in the preparation of the initial butenylbenzenes, the two methods are different and complementary to each other.

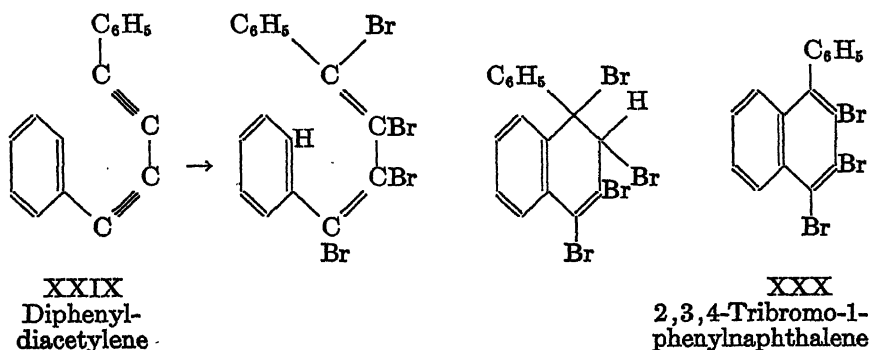
Another variation of the above cycloisomerization is provided by the Darzens (44) synthesis of naphthalene- and phenanthrene-carboxylic acids from benzylallyl- and naphthomethylallyl-acetic acids, respectively, which are isomerized under the influence of concentrated sulfuric acid, most probably by way of the isomeric propenyl compounds which contain the butenylbenzene system; e.g.,



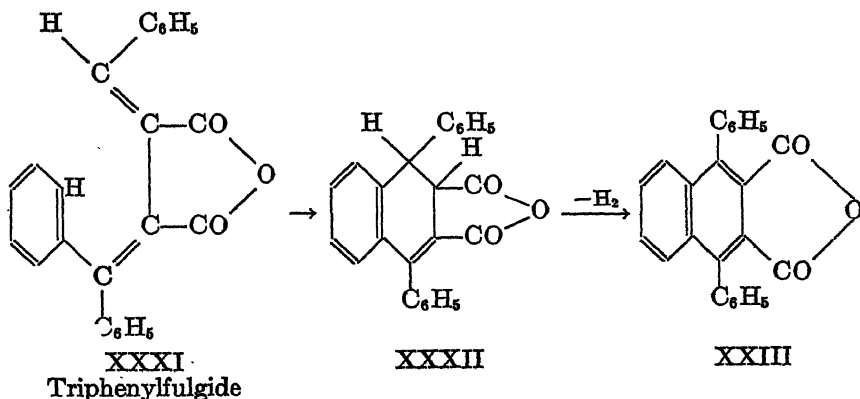
The migration of the double bond appears to be directed by the tendency to approach the carboxyl group.

C(a). Cycloisomerization of the phenylbutadiene system

A special instance of the cycloisomerization discussed in the preceding section is the case of the phenylbutadienes, in which the butenylbenzene system to be isomerized contains an additional (conjugated) double linkage. No such reaction is known for pure hydrocarbons (the pertinent case of 1,2,3,4-tetraphenylbutadiene is discussed on page 539), but undoubtedly the formation of 2,3,4-tribromo-1-phenylnaphthalene (XXX) upon the bromination of diphenyldiacetylene (XXIX) (85) must be formulated in the following manner, involving cycloisomerization of the primary tetrabromide and subsequent loss of one molecule of hydrobromic acid:



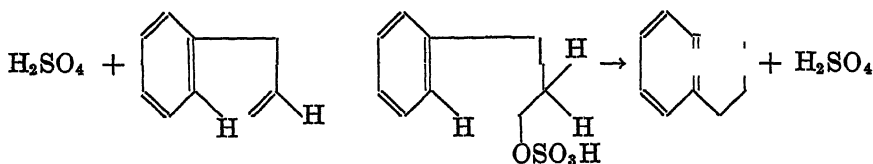
The same mechanism is involved in the photochemical isomerization of triphenylfulgide (XXXI) (84), which is catalyzed by iodine:



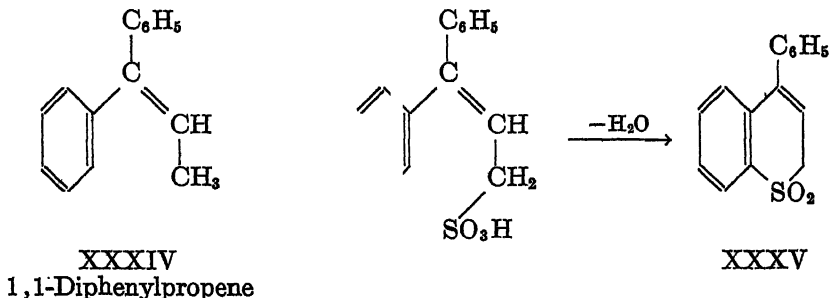
The intermediate dihydronaphthalene (XXXII) stage can be isolated if the photoisomerization is carried out in the absence of oxygen.

1. Mechanism of the isomerization

These isomerizations, which are usually brought about by reagents like concentrated sulfuric acid, aluminum chloride, stannic chloride (in the presence or the absence of hydrochloric acid), have been formulated so far—merely for the sake of simplicity—as involving the transfer of a hydrogen atom from the benzene nucleus to the central atom of the unsaturated side chain. It is quite possible that the actual mechanism of the reaction is addition of the catalyst (e.g., $\text{H}-\text{OSO}_3\text{H}$, $\text{H}-\text{HSnCl}_6$, etc.) to the double bond and splitting off of the catalyst molecule, the ortho hydrogen atom of the benzene nucleus now being involved:



Whether the additional hydrogen atom appearing in the newly formed ring is that of the aromatic nucleus or derives from the sulfuric acid may perhaps be decided in every case by using heavy sulfuric acid as the catalyst, but, even on the basis of the existing experimental evidence, it may be maintained that both mechanisms may actually occur (quite as in the similar case of dimerization by the transfer of hydrogen, not within the molecule but from one molecule to another). In the case of 1,2,3,4-tetraphenyl-1-butene, for example, it has been observed (22) that metallic sodium may produce the isomerization, which would seem to be a purely catalytic reaction. On the other hand, observations made in the case of 1,1-diphenylpropene (XXXIV) point definitely to a participation of the agent in the reaction. When concentrated sulfuric acid acts upon this hydrocarbon, a sulfur-containing compound³ of the formula $\text{C}_{15}\text{H}_{12}\text{O}_2\text{S}$ is obtained (9); according to its properties this compound has the structure of a thio- α -pyran derivative (4-phenyl-5,6-benzothio- α -pyran-1-dioxide) (XXXV) and owes its formation to the following reaction:

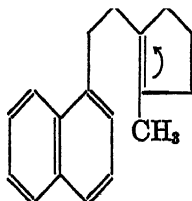


³ The substance has a melting point of 114°C . and on catalytic hydrogenation gives a dihydro compound melting at $169-170^\circ\text{C}$.

The above reaction resembles⁴ the course that bromination takes in the case of $(C_6H_5)_2C=C(CH_3)_2$, when the ω -derivative, $(C_6H_5)_2C=C(CH_3)(CH_2Br)$, is produced (91).

2. Influence of substituents on the cycloisomerization

Cook and his coworkers (33, 39, 40, 41, 43) have observed that a methyl group in the 2-position of the allyl group generally enhances the ring closure in the desired sense and suppresses side reactions, such as the formation of spiran derivatives. This is probably due to (a) a favorable valency deflection, caused by the more complete substitution on one side of the double bond, and (b) to the $+I$ effect of the methyl group, which causes an attraction of the nuclear protons by the neighboring unsaturated carbon atom, as shown in the following diagram:⁵

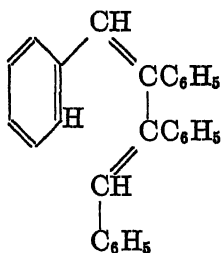


The influence of substituents in the aromatic nucleus on ring formation has never been studied, but it may be safely assumed that it is the same as for the cyclization reaction of phenylated fatty acids, as studied by v. Braun, Manz, and Reinsch (32); an unsubstituted phenyl nucleus reacts more easily than a *m*-tolyl system and less easily than a *p*-tolyl system (meta and para with regard to the hydrogen atom to be split off).

Also the question as to which of the two rings, cyclohexane or cyclopentane, is formed when the structural possibility for the formation of both is present has not yet been answered definitely. For the phenylated acids, Leuchs (60) and v. Braun and Manz (31) have shown that the six-membered ring is formed in preference to the five-membered ring, but in the hydrocarbon series the only case so far known points to the contrary. 1,2,3,4-Tetraphenylbutadiene (XXXVI) might cyclize to give either 1,2,3-triphenyl-1,4-dihydronaphthalene (XXXVII) or 1,2-diphenyl-3-benzylidenehydrindene (XXXVIII):

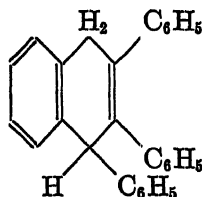
⁴ It is possible that the reaction consists in the well-known addition of the acid as $HO-SO_3H$, followed by dehydration and allylic rearrangement.

⁵ In the reaction of 2-methylcyclopentanone with β -(9-phenanthryl)ethylmagnesium chloride, the methyl group does not show this favorable effect (13; cf. 36, 51).

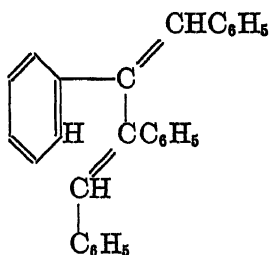


XXXVI

1,2,3,4-Tetraphenylbutadiene

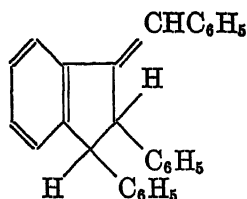


XXXVII

1,2,3-Triphenyl-1,4-dihydro-
naphthalene

XXXVI

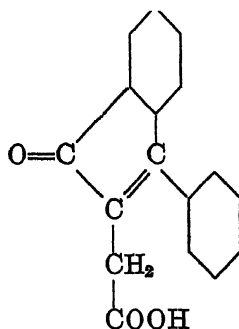
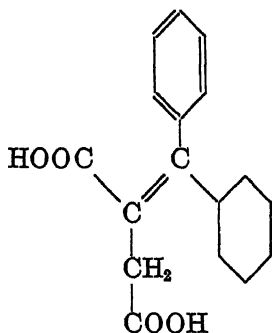
1,2,3,4-Tetraphenylbutadiene



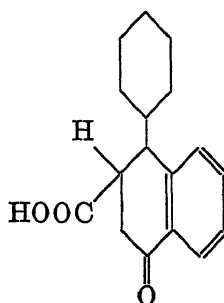
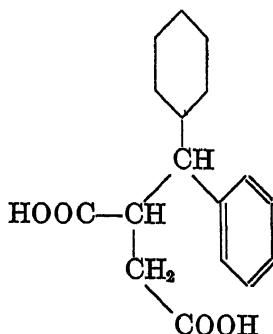
XXXVIII

1,2-Diphenyl-3-benzylidene-
hydrindene

Actually, only the indene derivative is obtained (22). It may be that steric influences—not yet clear—play a certain part in these cyclization processes. This view is supported by the following general observation (34): The diarylitaconic acids, on cyclization, form indene derivatives, a change which involves the α -carboxyl group,



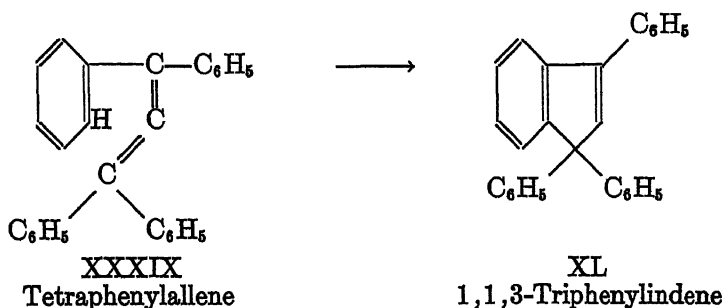
while the corresponding hydrogenated diarylmethylsuccinic acids, on dehydration, yield α -aryltetralones with the β -carboxyl group:



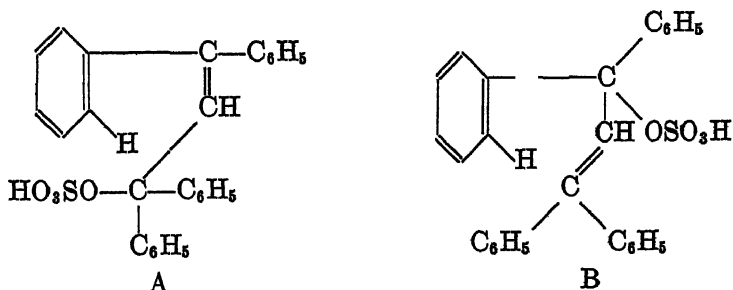
In the unsaturated acid, obviously, the aryl and the α -carboxyl groups are sterically close to each other, and, owing to the rigidity of the ethylene system, five-membered ring formation is preferred to the other possible cyclization, which is not preformed in the same sense.

D. Cycloisomerization of arylated allenes

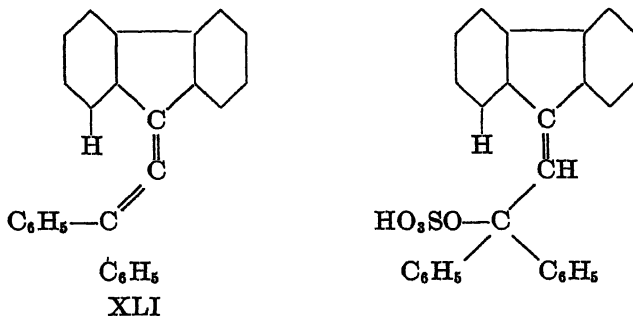
The isomerization of allenes into indenenes presents a very clear-cut case. Tetraphenylallene (XXXIX) is isomerized by strong acids into 1,1,3-triphenylindene (XL) (58, 89), according to the following scheme:



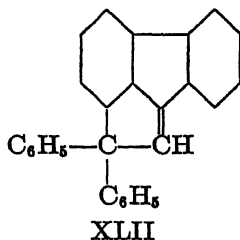
One assumes, in general, that the deeply colored halochromic compound which is formed and disappears in the course of this reaction is the intermediate A, from which the acid is split off again, removing the ortho



hydrogen atom from the benzene nucleus. This is, however, not in accordance with the behavior of 1,1-diphenyl-3-diphenyleneallene (XLI) (56). As the diphenylmethyl system seems to be more basic than the fluorenyl group, one would expect the occurrence of the following reaction:

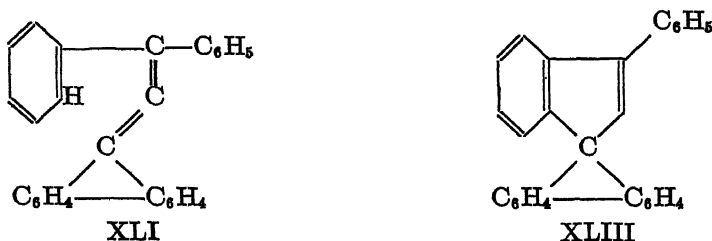


1,1-Diphenyl-3-diphenyleneallene



However, this reaction does not occur, possibly owing to the difficulty^a of formation of such a condensed ring system.

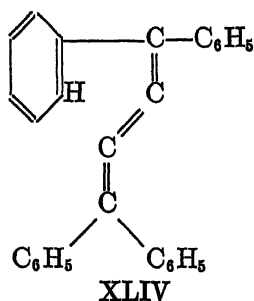
There is, however, another possibility. The addition of the acid to the allene system, yielding the halochromic compound, may have nothing to do with the cycloisomerization, but may only enhance this reaction by giving the rigid allene derivative a mobility which is required for the transition into a state which is geometrically apt to undergo cyclization. The intermediate product would therefore be of type B, the cycloisomerization of allene would be a special case of that of allylbenzenes, and the isomerization of 1,1-diphenyl-3-diphenyleneallene (XLI) would follow another course:



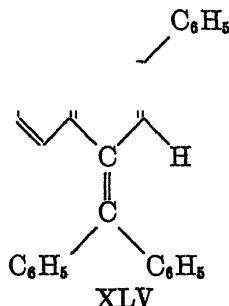
^a This difficulty has already been pointed out by v. Braun and Anton (30); recently, however, Koelsch (55) has actually isolated a substance of this complicated type.

Formula XLIII shows the correct structure of the final product. A decision between the two explanations is not yet possible.

Many similar cycloisomerizations have been observed (15, 92, 93), but the structure of the isomerization product has been elucidated only in the case of the dimeric triphenylallene (86) and of tetraphenylbutatriene (XLIV) (29), which gives 1-benzhydrylidene-3-phenylindene (XLV) according to the following scheme:

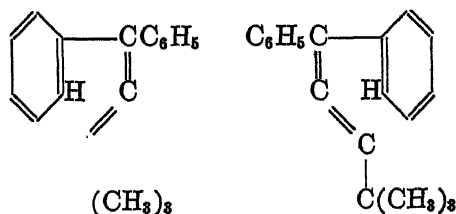


Tetraphenylbutatriene



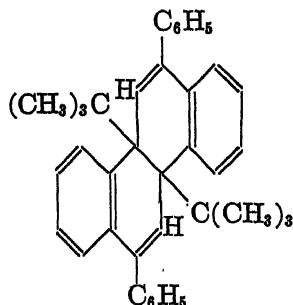
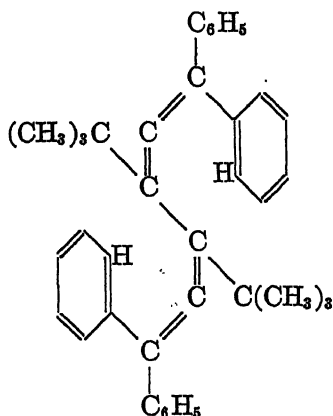
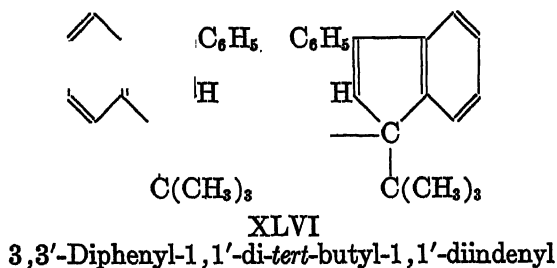
1-Benzhydrylidene-3-phenylindene

The arylated diallenes, obtained by Marvel and coworkers (47, 48, 62, 66, 83, 88; see page 531), are also apt to isomerize, but it is not yet clear which course this cycloisomerization takes. For example, the product obtained from 1,1,6,6-tetraphenyl-1,3-di-*tert*-butyl-1,2,4,5-hexatetraene (VIII) under the influence of mineral acids or by treatment with metallic sodium and subsequent removal of the sodium from the organometallic compound formed is not 3,3'-diphenyl-1,1'-di-*tert*-butyl-1,1'-diindenyl (XLVI), which might be expected according to the above schemes, as it does not show any tendency to dissociate into free radicals (49, 50), but it is also not 2,8-diphenyl-6b,12b-di-*tert*-butyl-6b,12b-dihydrochrysene (XLVII), as has been shown by Marvel and Peppel (62) on the basis of spectroscopic evidence.



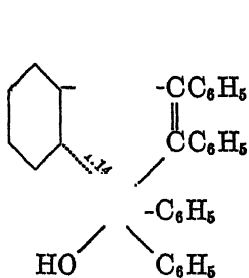
VIII

1,1,6,6-Tetraphenyl-1,3-di-*tert*-butyl-1,2,4,5-hexatetraene

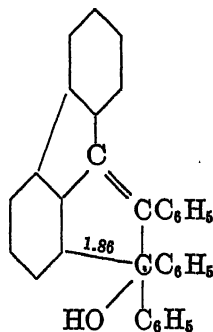


XLVII

An interesting example of the effect of steric influences on cyclization reactions of this type has been studied by Koelsch (54). Pentaphenylallyl alcohol (XLVIII) splits off water to give 1,1,2,3-tetraphenylindene, while the analogous 3-diphenylene-1,1,2-triphenylallyl alcohol (XLIX) is unable to do so, but behaves as 2,3,3-triphenyl-1-diphenyleneallyl alcohol, giving 1-diphenylene-2,3-diphenylindene.



XLVIII
Pentaphenylallyl alcohol

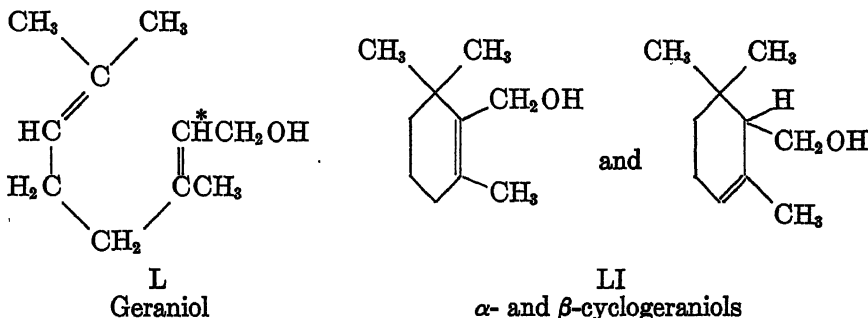


XLIX
3-Diphenylene-1,1,2-triphenylallyl alcohol

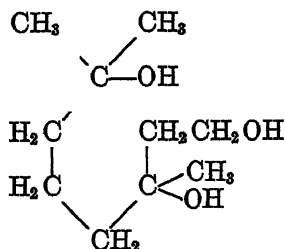
On the basis of certain assumptions, Koelsch calculates that the distances between the carbinol carbon atom and the nuclear carbon atom which are involved in the cyclization reactions are 1.14 and 1.86 Å., respectively, for the two different cases.

E. Cycloisomerization of terpene compounds

It is to be expected that the tendency toward cycloisomerization would not be confined to a convenient unsaturated chain linked with an aromatic nucleus, but that the latter could be substituted by other suitable olefinic systems. In this way doubly unsaturated chains, especially in the terpene group, are likely to form ring compounds possessing one remaining double bond. Geraniol (L), for example, is converted into α - and β -cyclogeraniols (LI),



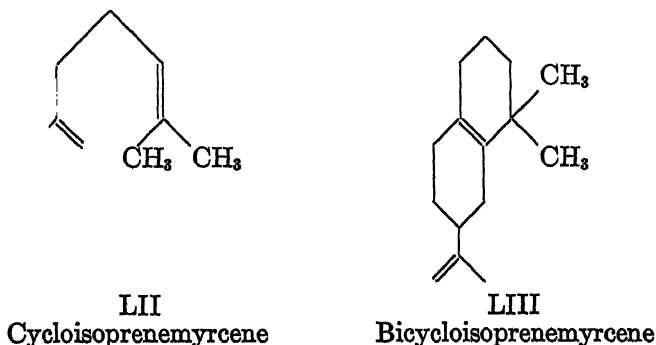
and the same is true of the corresponding aldehyde (citral) and the corresponding acid (geranic acid). The formation of the α -compound, which involves not only the migration of the hydrogen atom marked with an asterisk, but also that of the double bond, may be due to a partial allylic rearrangement during or after cyclization—or perhaps to the fact that the cycloisomerization, under the influence of strong acids, is preceded by hydration processes and is actually a “cyclodehydration”, e.g., of a compound like



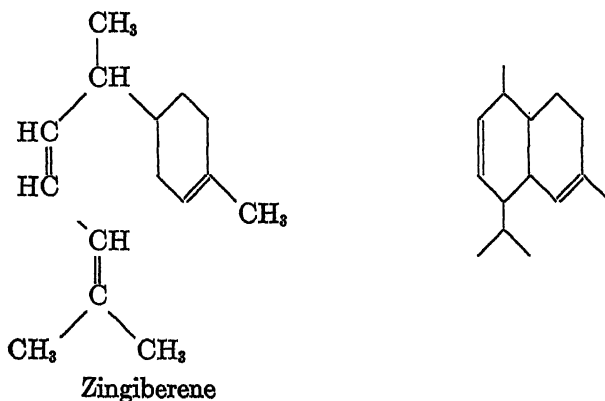
in which the final dehydration can give rise to both the α - and the β -forms.

In the same way hydrocarbons may be cyclized. The heterodimeriza-

tion product of isoprene and myrcene, the so-called cycloisoprenemyrcene (LII), is isomerized by means of strong acids to form bicycloisoprenemyrcene (LIII) (73, 79),



in which the location of the double bonds is somewhat arbitrary. This reaction parallels the Bogert synthesis of hydrogenated naphthalene derivatives (see page 535). Even cases similar to the Darzens synthesis are known, involving the migration of the double bond in the initial product before cyclization; for example, in the isomerization of zingiberene (75):

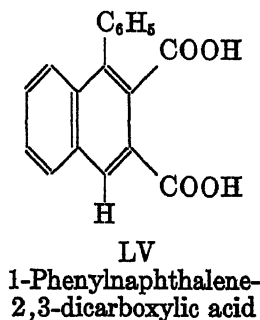
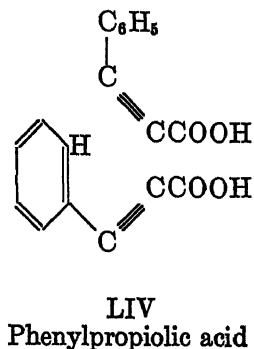


This cycloisomerization is energetically so favored that it sometimes occurs even in the absence of acid, e.g., under the conditions of dehydrogenation, under which zingiberene yields cadalene (1,6-dimethyl-4-isopropynaphthalene) (72, 74).

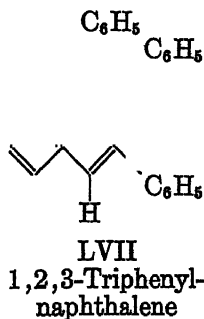
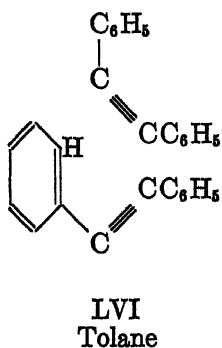
F. Bimolecular cycloisomerization of phenylacetylenes and related reactions

It is obvious that phenylacetylene (like phenylethylene) cannot undergo simple cycloisomerization, as this would lead to benzocyclobutadiene deriv-

atives. For a long time, however, it has been known (63) that two molecules of phenylpropionic acid (LIV) are apt to combine to yield (the anhydride of) 1-phenylnaphthalene-2,3-dicarboxylic acid (LV) under the influence of such reagents as acetic anhydride.

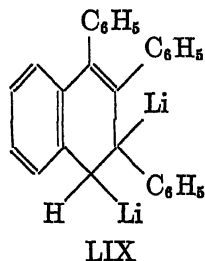
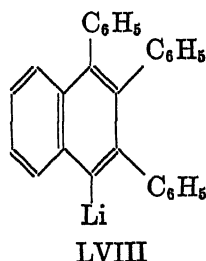


This reaction appears to reflect the existence of a certain molecular affinity between two molecules of the acetylenic compound. Recently the analogous reaction of tolane (diphenylacetylene) (LVI) was observed by Schlenk and Bergmann (76). When tolane was treated with metallic lithium in the presence of ether, various derivatives of 1,2,3-triphenylnaphthalene (LVII) were obtained.

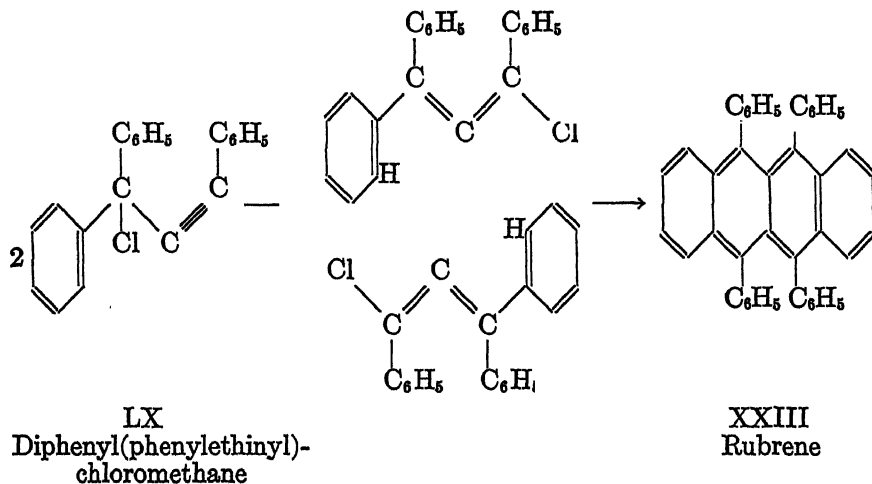


In spite of additional research (10, 22, 23), the intrinsic mechanism of this reaction has not become quite clear. Bergmann and Zwecker (23) put forward the suggestion that a dilithiostilbene, $\text{C}_6\text{H}_5\text{CLi}=\text{CLiC}_6\text{H}_5$, is formed at first and then reacts with a molecule of unchanged tolane. There are many possibilities of explaining on this basis the formation of the two organometallic compounds (LVIII and LIX) assumed to be present in the solution obtained from lithium and tolane but, whatever the intimate

mechanism of the reaction, the similarity in the behavior of phenylpropionic acid and of tolane is evident.



An equally surprising case of dimolecular cyclization is the formation of rubrene (XXIII) from diphenyl(phenylethynyl)chloromethane (LX), although this reaction is not actually an isomerization. According to Dufraisse (45; cf. 81) the chloromethane reacts in its isomeric form (for similar rearrangements see page 530), as follows:



Here again, a peculiar intermolecular interaction—between the two central carbon atoms—appears to play an important rôle.

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DIPOLE MOMENTS AS A TOOL IN THE DETERMINATION OF STRUCTURE

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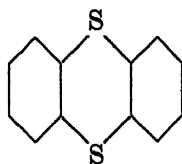
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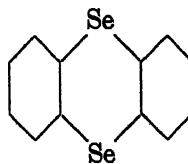
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I. INTRODUCTION

The determination of dipole moments has contributed very much to the final settlement of problems which arose in classical organic chemistry and to a more intimate knowledge of organic compounds. This has been possible because of the relatively simple correlation between the molecular electric moment and the electric moments of the single links within the molecule, which permits a sufficiently safe evaluation of the experimental figures. The method of dipole moments has even led to conclusions more or less unexpected from the classical point of view; e.g., it has proved that substances like thianthrene (13, 29, 34a, 49, 155, 176) or selenanthrene



Thianthrene



Selenanthrene

(49, 176) are not planar, but have a folded structure; in certain cases this surprising asymmetry has led to the isolation of enantiomorphic forms of quite unknown character (14, 50, 102a, 117, 177). In the following pages, some of the problems which have been or might be solved by means of dielectric measurements are reviewed. Following the lines on which the work in the Daniel Sieff Research Institute is proceeding, the discussion

deals mainly with the use of dipole moments for the solving of stereochemical questions, but, in addition, the detection of zwitter-ion structure in organic molecules by means of the new and simple physical tool of electric moment determinations is considered.

II. DETERMINATION OF STRUCTURE BY MEANS OF DIPOLE MOMENTS

It is known and need not be reëmphasized here that the *o*-, *m*-, and *p*-disubstituted benzene derivatives are characteristically differentiated by their dipole moments. Hassel and Naeshagen (97) have shown that this also applies to more highly substituted benzene compounds. This is true more especially for those substituents for which the valency linking the substituent to the nucleus coincides with its axis of symmetry,—atoms and groups like CH₃, CCl₃, NO, NO₂, NC, NCO, NSO, NCS, CN, and N₃,—while in other cases,—substituents like OR, SR, COOR, CHO, CONH₂, SCN, SO₃H, and derivatives,—the situation is much more complicated, owing to the additional degrees of freedom, so that here no absolutely decisive conclusions with regard to the relative position of substituents can be drawn from the dipole moments of the compounds.

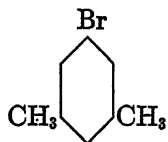
As the structures of practically all the simple di- and poly-substituted benzene derivatives have long been known, the method of dipole moments has not revealed many facts new to the organic chemist.¹ A few examples may show, however, that the method can be easily applied to more complicated cases of this kind and will certainly prove useful in the future.

(1) In an attempt to oxidize 3,5-dimethylbromobenzene (I) (12) with dilute nitric acid, there was obtained, in addition to the expected 3-methyl-5-bromobenzoic acid (II), a neutral product, the analysis of which pointed to the formula C₈H₈O₂NBr of a nitro derivative of I (15)². Of the two possible formulas (III and IV) the dipole moment ($\mu = 4.00$) decided in favor of III³. The substance described by Noelting, Braun, and Thesmar (131) as 5-bromo-4-nitro-1,3-dimethylbenzene and melting at 39–40°C. was therefore an impure specimen of the above compound, as its mode of preparation appears to be unambiguous.

¹ Also some important conclusions as to the mutual interactions of ortho substituents.

² The substance boiled at 171°C. at 52 mm. and solidified spontaneously. It crystallized from light petroleum in fine needles melting at 53°C. Calculated for C₈H₈O₂NBr: C, 41.7 per cent; H, 3.5 per cent; N, 6.1 per cent; Br, 34.8 per cent. Found: C, 41.3 per cent; H, 3.7 per cent; N, 6.4 per cent; Br, 35.2 per cent.

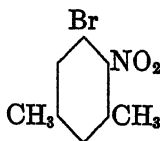
³ The moment of 2-nitrobromobenzene is 3.98; that of 4-nitrobromobenzene is 2.45 (120).



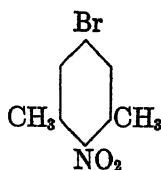
I
3,5-Dimethyl-
bromobenzene



II
3-Methyl-5-bromo-
benzoic acid



III



IV

(2) Guareschi and Biginelli (82) obtained, by the chlorination of 1-bromonaphthalene and by the bromination of 1-chloronaphthalene, a chlorobromonaphthalene, m.p. 119–119.5°C., to which they ascribed—without experimental proof—the structure of the 1,8-compound. As Bergmann and Hirshberg (26) have obtained the real 1-chloro-8-bromonaphthalene (m.p. 94–95°C.) (100) in an unambiguous way (from 1-chloro-8-naphthylamine) and as its dipole moment (2.64) is in accord with this structure, the substance of the Italian authors must have another structure; it may be the otherwise unknown 1,5-compound, which should have a very small dipole moment.

(3) The nitration of β -acetnaphthalide affords, according to Saunders and Hamilton (142), three isomeric nitro-2-naphthylamines. Their structures can be determined not only by purely chemical methods, but also by their conversion into the corresponding nitro-2-halogenonaphthalenes⁴ and the determination of their dipole moments. We have proceeded in this way (15). The nitro-2-bromonaphthalene (m.p. 191°C.) corresponding to the nitronaphthylamine melting at 203°C. has a dipole moment of 2.87 and is therefore the 6-nitro compound.⁵

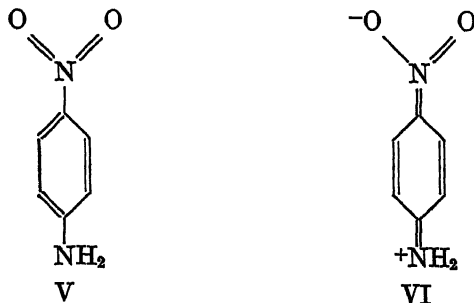
A second and perhaps more interesting problem, which can be solved by dipole moment measurements, is that of the fine structure of certain aromatic substances. (The analogous feature in the urea series has been discussed by Bergmann and Weizmann (34)). It was made clear some time ago (101) that the classical formulas for substituted benzenes are not fully adequate, since, by interaction between the substituents or between the substituent and the nucleus, electrons are shifted within the molecules. Although such an electronic shift will rarely develop into an electron transfer and the formation of a zwitter ion, our problem can be formulated thus: How great is the tendency of a given molecule to undergo transition

⁴ As will be shown later, the nitronaphthylamines exhibit certain peculiarities which make their use for this purpose somewhat difficult.

⁵ The 2-bromo-1-nitronaphthalene and the 2-bromo-8-nitronaphthalene which might have been formed would have moments of approximately 4.1 and 3.29, corresponding to the *o*- and *m*-bromonitrobenzenes.

to the zwitter-ion state? This formulation makes it clear that the determination of dipole moments can give an important clue to the question, since zwitter ions have an unusually high dipole moment ($4.774 \times$ the distance of the charges). If too high a dipole moment is observed for a given molecule, one will have to consider the possibility of (a) part of the molecules being in a zwitter-ion state and part behaving "normally," or of (b) all molecules having undergone an electron shift towards the zwitter-ion formula, representing therefore an intermediate resonance state between this and the classical formula. Dipole moments by themselves do not permit a decision between the two alternatives, but other physical evidence (see, e.g., page 560) makes it probable that the second alternative is correct. In the following pages a brief review is given of the pertinent cases known so far.

(1) The moment of *p*-nitroaniline ($\mu = 6.18$ to 7.1)⁶ is higher than the sum of the moments of the two substituents ($\mu = 5.4$). As this sum is the upper limit for a normal *p*-disubstituted benzene, the structural formula V is unsatisfactory and a shift towards VI becomes likely (171).⁷



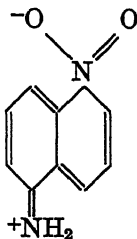
The same is true for *p*-nitrodimethylaniline ($\mu = 6.87$) (126), *p*-nitrodi-phenylamine ($\mu = 5.82$) (102), and 4-nitro-4'-aminodiphenyl ($\mu = 6.46$). An important argument that this effect is due to a resonance phenomenon has been put forward by Birtles and Hampson (36; cf. 155a, 201). Resonance between two forms can occur only when the atoms concerned remain in practically the same position in both states (152). In a molecule such as nitroaminodurene the plane of the nitro group is, for steric reasons, perpendicular to the benzene ring, while in the form corresponding to formula VI it must be coplanar. As this prevents resonance, nitro-

⁶ The moments quoted are taken from the list of dipole moments given in *Transactions of the Faraday Society* 30, (1934). 4-Aminodiphenyl has a slightly higher moment (1.71; unpublished results, see table 12) than aniline. This may be due to an effect analogous to that in the case of *p*-nitroaniline, owing to the peculiarities of the diphenyl structure, discussed on page 576).

⁷ In view of the many factors involved, it is not clear whether the same effect occurs in *o*-nitroaniline ($\mu = 4.45$ (98); $\mu = 4.25$ (178)) and in *o*-nitrodiphenylamine ($\mu = 4.13$).

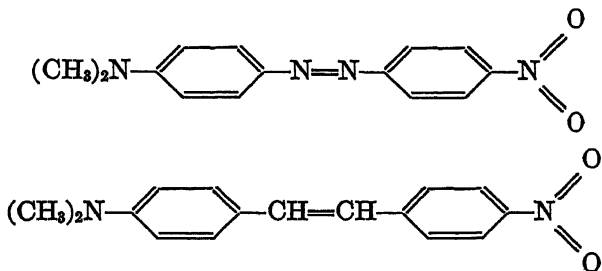
aminodurene should not show the extreme dipole moment of 4-nitro-aniline, and this is actually the case ($\mu = 4.98$).

Whether similar effects occur in the naphthalene series is not clear. According to Vasilieff and Syrkin (186),⁸ 1-nitro-5-naphthylamine has a moment of 5.22, which is practically equal to the sum of the moments of the substituents. It may be noted that 1-nitro-5-naphthylamine could assume the quinoid formula

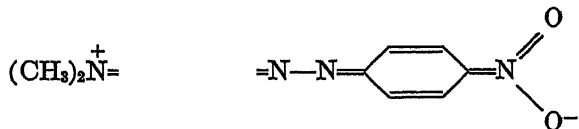


corresponding to II; this structure would represent the system of the unknown 1,5-naphthoquinone, of which derivatives have been prepared (35, 201).

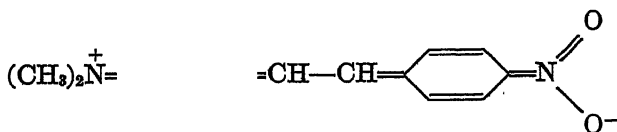
It is clear that other instances may still be found. Thus, A. Weizmann (195) has observed that 4-nitro-4'-dimethylaminoazobenzene and 4-nitro-4'-dimethylaminostilbene



have moments as high as 8.1 and 8.3, owing to forms such as



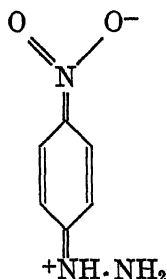
and



⁸ The remarks made above apply to the moments (4.89; 4.47) reported by them for 2-nitro-1-naphthylamine and 1-nitro-2-naphthylamine, respectively.

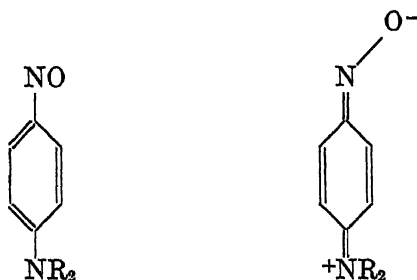
contributing to the resonance state of the molecules. The same conclusion has been drawn recently from the spectra of such stilbene derivatives and from their inability to form stable geometrical isomers (48).

(2) 4-Nitrophenylhydrazine has, according to Ulich and coworkers (183), a dipole moment of 7.2. This is due to a shift towards the structure



which will also occur in the 2,4-dinitro- and 2,4,6-trinitro-phenylhydrazines ($\mu = 5.8$ and 4.40 , respectively).

(3) One of the first anomalies observed (112) was that of the *p*-amino-nitrosobenzenes:

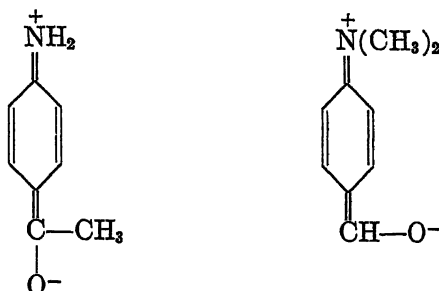


4-Nitroso- <i>N</i> -methylaniline	7.38
4-Nitroso- <i>N</i> -dimethylaniline	6.90 (in benzene)
4-Nitroso- <i>N</i> -diethylaniline	7.18 (in benzene)

The formula to be postulated is not only formally parallel to the accepted quinone monoxime structure of *p*-nitrosophenol, but it is also in agreement with the chemical behavior of the above substances (easy hydrolysis to quinone monoxime; addition of methyl iodide and of benzoyl chloride at the ends of the charged system), as has been pointed out by E. Bergmann and A. Weizmann (30, 32).

(4) The splitting of the double bond in the carbonyl (and the carboxyl) group is a feature which has been assumed previously. Hence no difficulty arises in ascribing the dipole moment (4.29) (97) of 4-aminoaceto-

phenone and that (5.6) of 4-dimethylaminobenzaldehyde to a shift towards the following structures (194)



and in explaining on similar lines the dielectric behavior of ethyl and methyl 4-aminobenzoates (moments of 4.0 and 3.3) (71) and of 4-amino-

TABLE I

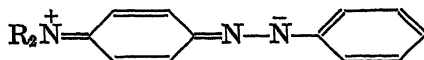
SUBSTANCE		
4-Dimethylaminocinnamaldehyde.....	$(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{CH}=\text{CHCHO}$	5.4
4-Dimethylaminobenzylideneacetone..	$(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{CH}=\text{CHCOCH}_3$	5.3
4-Dimethylaminocinnamylideneacetone, m.p. 120°C.....	$(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{CH}=\text{CHCH}=\text{CHCO-CH}_3$	6.7
4-Dimethylaminobenzylideneacetophenone.....	$(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{CH}=\text{CHCOC}_6\text{H}_5$	4.4
4-Dimethylaminocinnamylideneacetophenone.....	$(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{CH}=\text{CHCH}=\text{CHCO-C}_6\text{H}_5$	5.4
Ethyl 4-dimethylaminocinnamate.	$(\text{CH}_3)_2\text{NC}_6\text{H}_4\text{CH}=\text{CHCOOC}_2\text{H}_5$	4.7

benzamide ($\mu = 4.7$) (61). The Raman spectra of the benzoic acid esters lead to similar conclusions (131). A. Weizmann (194) has shown recently that the same effect is still more marked when the distance between the amino and the carbonyl groups is increased by the interposition of vinylene groups. This may be concluded from table 1.

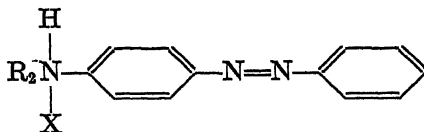
It has also been suggested that the intense colors which the unsaturated carbonyl compounds listed in table 1 develop on contact with concentrated sulfuric acid are due to fixation of the elements of the acids to the ends of the charged chain, as is the case with the aminoazo dyes (see below).

(5) *Aminoazo dyes*. E. Bergmann and A. Weizmann (30, 32) have observed that aminoazo dyes such as 4-aminoazobenzene ($\mu = 2.71$), 4-dimethylaminoazobenzene ($\mu = 3.68$), 4-benzeneazo-1-naphthylamine ($\mu = 2.50$), and 1-benzeneazo-2-naphthylamine ($\mu = 2.14$) have dipole

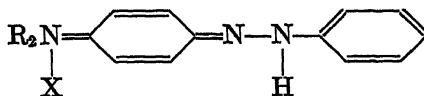
moments much higher than expected; as azobenzene (see page 578) has no finite dipole moment, the dipole moments of these azo dyes, in their classical formulas, should be those of aniline ($\mu = 1.55$) and of the α - and β -naphthylamines ($\mu = 1.44$; $\mu = 1.74$), respectively. All must therefore be formulated as follows:⁹



This formula agrees with the behavior of the aminoazo dyes towards acids. Hantzsch (88, 89, 90, 103) has discovered that they form two series of salts, yellow and violet ones, which have been formulated by the author¹⁰ as



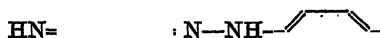
and



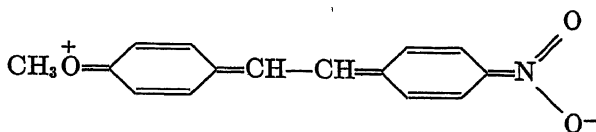
and which would be formed by the addition of HX to the normal and to the zwitter-ion formula, respectively. Furthermore, it is important that dimethylaminoazobenzene has a spectrum which is different from that of azobenzene, while that of its yellow hydrochloride coincides completely with the spectrum of azobenzene (90). This shows at the same time that we must assume that all of the molecules have undergone the electron shift towards the zwitter-ion formula; otherwise, one would expect that the spectrum of the aminoazo dye would be composed of the azobenzene spectrum and a superimposed second one corresponding to the zwitter-ion molecules.

(6) *Methoxy compounds.* It is clear that the effects discussed above will not be restricted to amino compounds, but will apply to those substituents in which unshared electrons are present, e.g., in hydroxy and alkoxy compounds. For 4-nitro-4'-methoxystilbene and 4-nitro-4'-methoxyazobenzene, this has been postulated on the basis of spectroscopic evidence (75), and recent experiments by A. Weizmann (195) confirm this assumption; only an electron shift towards the structures

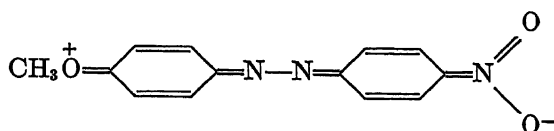
⁹ In the case of the non-methylated azo dyes a transfer of hydrogen would be possible, leading to a quinone imine phenylhydrazone structure,



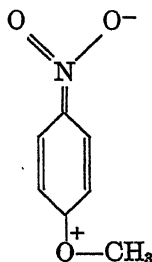
but this would place the amino and the dialkylamino compounds in two different classes, which is certainly not justified.



and

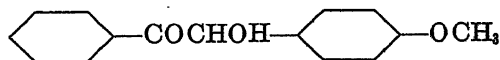


would explain the dipole moments (7.8 and 6.5) observed. Even in simpler molecules dielectric indications to the same effect are already known, although they are not very marked. 4-Methoxyazobenzene, for example, has a moment slightly greater than that of anisole ($\mu = 1.29$ (30, 32, 189) as compared with 1.16 and 1.23 (64, 98, 189)). Similarly, the anomaly that the dipole moment falls from *o*-nitroanisole to *m*-nitroanisole, but rises again to *p*-nitroanisole (57) will be rightly ascribed to a shift in the latter substance towards

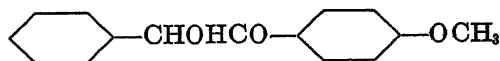


For *o*-hydroxyazo dyes, a zwitter-ion formula has been temporarily postulated by R. Kuhn (108), but their dipole moments show no indication to that effect (30, 32). *p*-Hydroxyazobenzene has in benzene solution a moment (1.62) identical with that (1.61) of phenol (63), but in dioxane solution a markedly higher one (2.04). Whether this is due to a shift towards the zwitter-ion formula or to a tautomeric change to a quinone hydrazone structure remains undecided.

An interesting illustration of the problem discussed is provided by the difference in the stability of anisbenzoin and of benzanisoin:



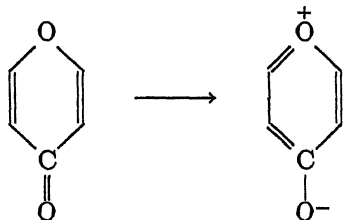
Anisbenzoin



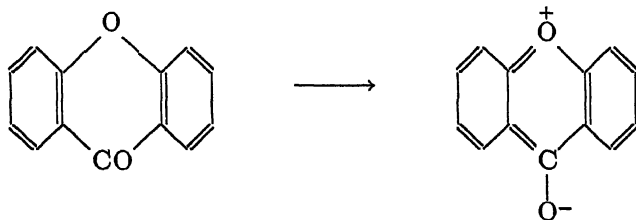
Benzanisoin

The latter is more stable because of the interaction between the methoxyl and carbonyl groups through the benzene ring, which is made impossible in anisbenzoin by the interposition of the CHOH group (11).

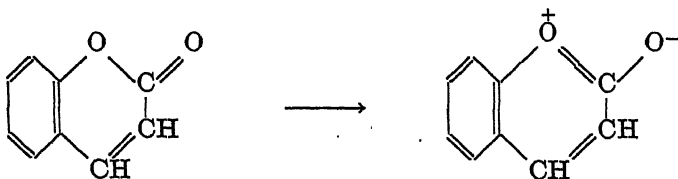
(7) In a less definite form, the conclusion that the classical formula must be replaced by a zwitter-ion structure has been drawn previously in the γ -pyrone series in order to explain certain chemical peculiarities (30, 32): the reaction of γ -pyrone with methyl iodide and with methylmagnesium iodide, both of which add to the ends of a charged system,



and the inactivity of pyrones and xanthenes

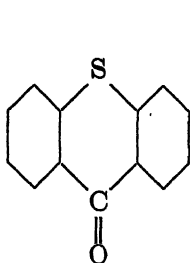


towards certain carbonyl reagents. The dipole moments of 2,6-dimethylpyrone ($\mu = 4.05$ (99), 4.48 (137), 4.6 (111), 4.62 (185)), 2,6-diphenylpyrone ($\mu = 3.82$ (99, 111)), and xanthone ($\mu = 3.07$ (30, 32), 3.0 (111), 2.9 (137, 185), 3.11 (110)) are higher than the difference between those of the carbonyl group and of the ether oxygen ($3.05 - 1.13 = 1.92$), which again points to a zwitter-ion formula. The same is true for 2,6-diphenylthiopyrone, of which the moment is 4.39 (4), while the difference between the dipole moments of the carbonyl and the sulfide is ($3.05 - 1.51 =$) 1.54. In the same way, the excess in the moment of coumarin and of 3-phenylcoumarin (111, 136, 185) over the theoretical value (resultant from the $\text{C}-\text{O}-\text{C}$ moment and the $\text{C}=\text{O}$ moment interacting at about 60°) is to be explained by a shift according to the scheme

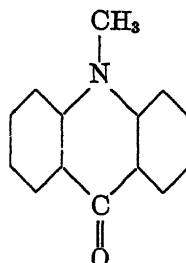


by which—as in the case of xanthone—a more fully aromatic (naphthalenoid) structure is produced. Analogous conclusions have been drawn from the Raman spectra of these substances by Volkenshtein and Syrkin (187).

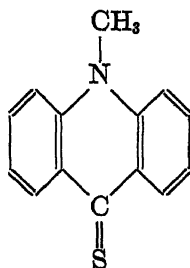
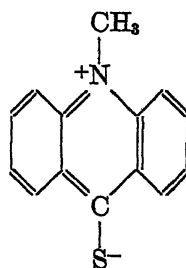
These observations have recently been extended by A. Weizmann (195) to include thioxanthone and *N*-methylacridone ($\mu = 5.4$ and 3.5, respectively).



Thioxanthone

*N*-Methylacridone

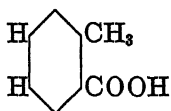
This effect is still more pronounced for the corresponding thioketones,—xanthione, thioxanthione, and *N*-methylthioacridone,—of which the dipole moments are 5.4, 5.2, and 5.2, respectively. These measurements, however, do not indicate what Gleu and Nitzsche (79) have postulated recently on the basis of chemical evidence, namely, that *N*-methylthioacridone exists exclusively in the form of a zwitter ion:

*N*-Methylthioacridone

III. THE ETHYLENIC DOUBLE BOND

The methods used in classical organic chemistry for assigning configurational formulas to ethylenic isomers were mostly based on the comparison of certain physical properties, of which none, however, had a theoretically founded relationship to the geometrical structure. This is especially conspicuous in those cases where not all of the possible geometric isomers are preparatively accessible. One of the methods (A. Werner (197)), however, may be mentioned, as it resembles the application of dipole moment measurements. The *cis*-isomers are compared with the

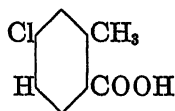
ortho compounds and the *trans*-isomers with the para compounds containing the same substituents: since *o*-toluic acid has a lower melting point than the para compound and 4-chloro-2-methylbenzoic acid a lower one than 4-methyl-2-chlorobenzoic acid, it is concluded that the lower melting forms of crotonic acid and β -chlorocrotonic acid are the *cis*-forms (with regard to the relative positions of the methyl and carboxyl groups).



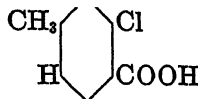
o-Toluic acid
(m.p. 102°C.)



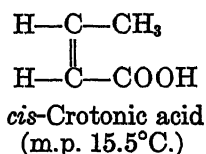
p-Toluic acid
(m.p. 176°C.)



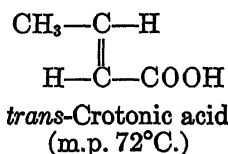
4-Chloro-
2-methylbenzoic
acid
(m.p. 130°C.)



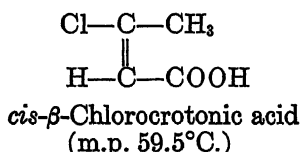
4-Methyl-
2-chlorobenzoic
acid
(m.p. 155°C.)



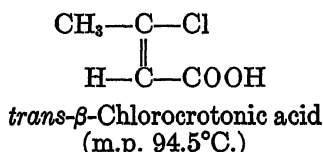
cis-Crotonic acid
(m.p. 15.5°C.)



trans-Crotonic acid
(m.p. 72°C.)



cis- β -Chlorocrotonic acid
(m.p. 59.5°C.)

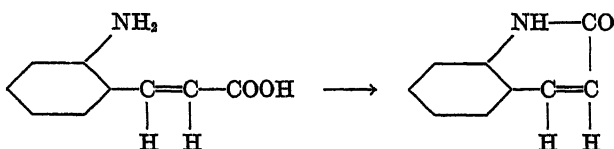


trans- β -Chlorocrotonic acid
(m.p. 94.5°C.)

It is clear that this rule cannot be generally valid, as the melting point is not a function of the geometrical structure of the molecule only. This criterion is, furthermore, inapplicable in cases where only one of the geometrical isomers is known.

The only exact method of configurational analysis known in classical organic chemistry was that based on ring-closure reactions. If, of two isomers, only one is capable of forming a ring between two substituents, it is clear—in view of the rigidity of the double bond—that these two substituents must lie on the same side of the plane of the double bond.¹⁰

¹⁰ Besides the much-quoted case of the pair maleic acid-fumaric acid, the *o*-amino-cinnamic acids may be recalled, only one of which is able to form a cyclic lactam and therefore is the *cis*-form (162):

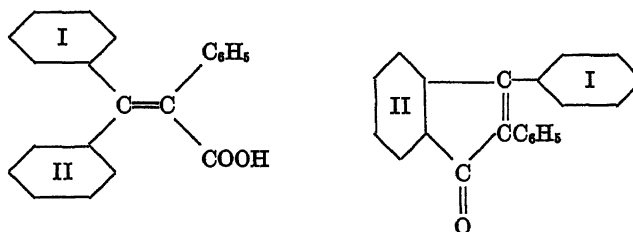


Analogously, Shriner and Sharp (151) have recently ascribed a *trans*-structure to

Even in those cases where both isomers are capable of forming the same cyclic product—one of them obviously by steric rearrangement, owing to the conditions of reaction—the different ease of reaction is indicative of the configuration of the starting materials: the form which is capable of cyclization without previous change in configuration will always cyclize more easily. It is evident that in such a case a conclusion can be drawn only when both isomers can be compared. A good example is furnished by the isomeric α,β -dibromocinnamic acids: one of them cyclizes under the influence of sulfuric acid at room temperature, the other one on distillation with phosphorus pentoxide, both to give the same 2,3-dibromoindone (78, 109, 139).¹¹

the *o*-hydroxybenzalacetone which is formed from the non-lactonizable *trans*-*o*-hydroxycinnamic acid and methylmagnesium iodide.

Another interesting example has been studied by Koelsch (105). The α,β -diphenylcinnamic acids are capable of forming 2,3-diphenylindones:



When rings I and II are different (differently substituted), the two isomers give different diarylindones, the structures of which give a lead to the assignment of configurational formulas to the isomeric acids: the ring forming the indone skeleton must have been near the carboxyl group, which appears as carbonyl in the resulting molecule. The following pairs have been studied: α,β -diphenyl- β -(*p*-methoxyphenyl)acrylic acids, α,β -diphenyl- β -(*p*-chlorophenyl)acrylic acids, and *cis*- α,β -diphenyl- β -(*p*-tolyl)acrylic acid.

¹¹ For the corresponding α,β -dichlorocinnamic acids, of which the isomer melting at 121°C. is cyclized easily, see Roser and Haselhoff (139). In other cases only one of the isomeric forms is cyclized, while the other one remains unchanged or is decomposed under the same conditions: α -chlorocinnamic acid (m.p. 110–111°C.) gives α -chlorotruxone (dimer of 2-chloroindone) (125); α -bromocinnamic acid, m.p. 120°C., behaves analogously (118, 124); α -methylcinnamic acid (m.p. 91–92°C.) (167); β -methylcinnamic acid (m.p. 131.5°C.) (165); α,β -dimethylcinnamic acid (39, 46).

For the sake of completeness, it may be noted that substitution reactions on ethylenic compounds can hardly be used for the determination of configuration, as it is not known whether they proceed with or without change of configuration. Thus, the conversion of the diiodoethylenes by means of potassium cyanide into maleic acid dinitrile and fumaric acid dinitrile, respectively, does not itself prove that the diiodo compounds have the same structure, although in this case it is true (104). The comparison of velocities of substitution is even less worthy of confidence, although it has often been used, as may be seen from the following examples: *cis*-

Dipole moments permit an exact determination of configuration in cases where the molecules contain at the two olefinic carbon atoms at least two polar substituents, even when only one of the geometrical isomers is known. In cases like the *cis*- and *trans*-cinnamic acids (or the crotonic acids) no decision can be made, as the dipole moments will be practically identical (17, 67, 81). The easiest case is again that of ethylenes sub-

TABLE 2

SUBSTANCE		REFERENCE
1,2-Dichloroethylene.....	1.74 <0.7	(128) (128)
1,2-Dibromoethylene.....	1.35 0	(69) (69)
1,2-Diiodoethylene.....	0.75 0	(68, 69) (68, 69)
1-Chloro-2-bromoethylene.....	1.55 0	(69) (69)
1-Chloro-2-iodoethylene.....	1.27 0.57	(70) (70)
1,2-Dichloro-1-hexene.....	1.99 0.57	(132) (132)
α,β -Dichlorostilbene, m.p. 144°C.....	0	(17)
m.p. 60°C.....	2.69	(17)
α,β -Dibromostilbene*, m.p. 68°C.....	2.53	(17)
α,β -Dinitrostilbene, m.p. 186°C.....	0	(17)
4,4'-Dinitrostilbene, m.p. 280-285°C.....	0	(17)

* The isomer melting at 206–208°C. was too insoluble to be measured.

stituted by single atoms or by groups for which the link with the olefinic carbon atom is the axis of symmetry. The pertinent examples are summarized in table 2.

dichloroethylene and iso- β -chlorocrotonic acid (probably the *cis*-form) lose, under the influence of alkali, one molecule of hydrogen halide more slowly than the *trans*-form (51, 76), while for dibromo- and diiodo-ethylenes the order is reversed (52, 53, 184). Similar conclusions obviously apply to the comparison of velocities of addition (43).

The *trans*-compounds are characterized by a dipole moment of 0 or by a small moment, the *cis*-forms by a finite electric moment. For 1-chloro-2-iodoethylene Errera (70) has assumed that the higher moment corresponds to the *trans*-form, supposing that the iodine atom is "positively charged with regard to the chlorine atom," i.e., that the direction of the C—Cl moment is not the same as that of the C—I moment, but this conclusion seems to be unfounded, as may also be seen from the comparison of the moments of, for instance, *o*- and *p*-bromiodobenzenes (188): the ortho compound, which corresponds very closely to the *cis*-form, has a higher moment (1.73; 1.86) than the para isomer ($\mu = 0.49$) (22, 72, 190).

TABLE 2A

SUBSTANCE	FORMULA	MELTING POINT	μ	REFER- ENCE
		°C.		
<i>p</i> -Chlorodiphenylvinyl bromide....	$\begin{array}{c} \text{ClC}_6\text{H}_4 \\ \quad \diagdown \\ \quad \text{C}=\text{CHBr} \\ \quad \diagup \\ \text{C}_6\text{H}_5 \end{array}$	94-95	2.27	(21)
		43-44	1.28	(21)
<i>p</i> -Bromodiphenylvinyl bromide....	$\begin{array}{c} \text{BrC}_6\text{H}_4 \\ \quad \diagdown \\ \quad \text{C}=\text{CHBr} \\ \quad \diagup \\ \text{C}_6\text{H}_5 \end{array}$	109	2.43	(21)
		42-44	1.22	(21)
<i>p</i> -Chlorotriphenylvinyl bromide....	$\begin{array}{c} \text{ClC}_6\text{H}_4 \\ \quad \diagdown \\ \quad \text{C}=\text{CBrC}_6\text{H}_5 \\ \quad \diagup \\ \text{C}_6\text{H}_5 \end{array}$	160	2.61	(17)
		119	1.57	(17)

A discussion of the absolute magnitude of the dipole moments of the *cis*-forms lies outside the scope of this review; it depends on the state of resonance between the classical and some other structural formulas (65, 153).

In table 2A a second group of dihalogenated olefins is listed, which shows a surprising feature. The *cis*-forms have the expected dipole moments, but their isomers have, instead of zero moment, a moment approximately half of that of the *cis*-configuration. E. Bergmann (17) has suggested that the compounds characterized by the lower moments are molecular compounds of the *cis*- and the (unknown) *trans*-forms and therefore show half of the dipole moment of the former. These combinations, therefore, resemble in a certain way the racemic compounds built up from a levo- and a dextro-rotatory form.

We feel sure that this new stereochemical effect is not restricted to the three pairs listed in table 2A, but that in many cases, e.g., in the series of the substituted cinnamic acids, similar conditions prevail. In the above-quoted paper of Koelsch (105), some indications to that effect may be found. Koelsch has observed that his substituted triphenylvinyl bromides, although appearing homogeneous, on successive reaction with magnesium and carbon dioxide give mixtures of the two isomeric corresponding diaryl-cinnamic acids. Theoretically, it is possible that such substitution reactions are accompanied by partial inversion of configuration, but it may well be that the apparently homogeneous triphenylvinyl bromides actually were molecular compounds of the possible geometrically isomeric forms.

In their extensive photochemical studies, Stoermer and coworkers (159, 160, 161) have observed some interesting cases in which the geometrical isomers of substituted cinnamic acids, on irradiation, produce "mixed forms": β -methylcinnamic acid (melting point of the mixed form, 76°C.); β -ethylcinnamic acid (m.p. 62–63°C.); *o*-methoxy- β -methylcinnamic acid (m.p. 76–78°C.); β -*p*-dimethylcinnamic acid (m.p. 94–96°C.) (compare Lindenbaum (119)); β -propylcinnamic acid (m.p. 59–60°C.). In the case of α,β -dimethylcinnamic acid the synthesis (141) yielded a solid and a liquid form. O. Blum-Bergmann (39), however, showed that, under suitable conditions, the two isomers (melting points 108.5–110°C. and 105–107°C.) can be obtained separately in crystalline form.

The tendency to form such molecular compounds seems somewhat characteristic for the whole group. We have observed that *trans*- α,β -dibromostilbene (m.p. 206–208°C.) gives with tolane a molecular compound (ratio 1:1; m.p. 195°C.), according to figure 1, and that in the bromination of tolane a molecular compound (ratio 1:2; m.p. 77°C.) is formed with *cis*- α,β -dibromostilbene (m.p. 68°C.) (150).¹² *cis*- α,β -Dichlorostilbene (m.p. 60°C.) and tolane give an analogous molecular compound (ratio 2:1; m.p. 67–69°C.).^{13,14}

¹² The substance was recrystallized from methyl alcohol as long needles and was identified by analysis. (Calculated for $C_{12}H_{10}Br_2$: C, 59.0 per cent; H, 3.5 per cent. Found: C, 59.3 per cent; H, 3.7 per cent.)

¹³ This substance was also recrystallized from methyl alcohol. Calculated for $C_{12}H_{10}Cl_2$: Cl, 21.0 per cent. Found: Cl, 20.9 per cent.

¹⁴ It may perhaps be surprising that in the above cases the "*cis*"-compounds have the higher melting point, but it must be recalled that the *cis*-structure refers to the two highly polar substituents in the molecule only, while the melting point may be influenced by the other substituents too. Furthermore, the melting point regularities need not be valid throughout, and it is interesting that Stoermer and Simon (166) have already observed that ultraviolet light converts the high-melting *p*-bromodiphenylvinyl bromide into the lower melting form. As they assigned, on the basis of the temperature of melting only, the *trans*-structure to the high-melting form, they saw in this conversion an irregularity, but actually here, as in other cases, ultraviolet light produces the reaction *cis* \rightarrow *trans*.

A second group of substituted olefins, in which dipole moments give a definite answer to the problem of configuration, has been assembled in table 3. Each of these substances contains at least one substituent not complying with the above definition,—namely, that the link should be the axis of symmetry for the substituent.

In the case of the two methyl β -bromocinnamates, it is clear that the ester of the lower melting acid (with the lower moment) is the *trans*-form,

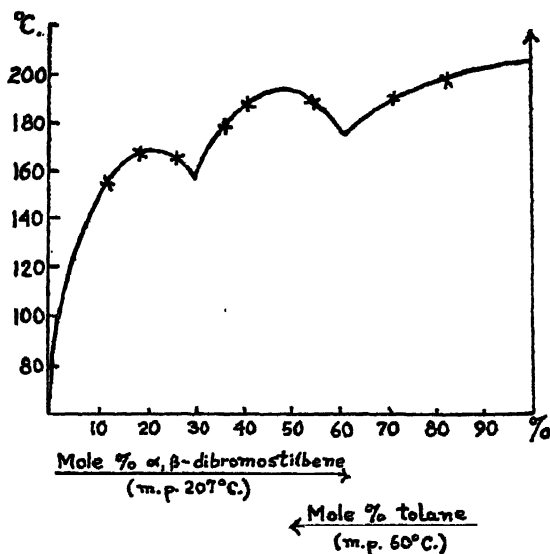
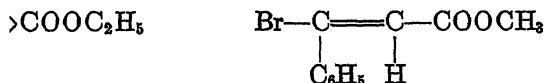


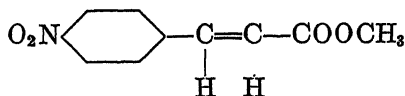
FIG. 1

all the more since, as would be expected, it has the same dipole moment as ethyl *p*-bromobenzoate ($\mu = 1.82$) (17).



By the same reasoning, ethyl 4-nitrocinnamate is the *trans*-compound, ethyl *p*-nitrobenzoate having a moment of 3.84 (17). This is understood, as ethyl *p*-nitrocinnamate is obtained by the nitration of *trans*-cinnamic acid. Methyl 4-nitro- α -phenylcinnamate will also contain the nitrophenyl and the carboxyl groups in the *trans*-position¹⁵:

¹⁵ Obviously, in the reaction between phenylacetic acid and *p*-nitrobenzaldehyde, the *trans*-isomer is formed, owing to the repulsion between the highly polar groups. This observation lends support to the theory of the Pschorr synthesis of phenanthrenes, advanced by Ruggli and Staub (140; compare 196).

Methyl 4-nitro- α -phenylcinnamate

That the dipole moment of the above compound is slightly higher than that of ethyl 4-nitrocinnamate corresponds to the fact (17) that ethyl cinnamate also has a slightly lower moment than methyl α -phenylcinnamate.¹⁶

The two β -bromocinnamic acids are formed by the addition of hydrobromic acid to phenylpropionic acid. In contradistinction to the findings of Sudborough and Thompson (170), we have observed that *cis*-addition is slightly favored. Stereochemically, it is interesting that debromination

TABLE 3*

SUBSTANCE	MELTING POINT	μ	REFERENCE
	°C.		
Methyl α,β -dibromocinnamate	100	2.68	(17)
	134	1.89	(17)
Methyl β -bromocinnamate	159	2.63	(17)
	130	1.81	(17)
(4-Bromobenzal)acetophenone	120	2.47	(17)
(4-Bromobenzal)-4'-bromoacetophenone	185-186	2.03	(17)
α,β -Dibromobenzalacetophenone	113-114	3.17	(17)
β -Bromobenzalacetophenone	B.p. 220° at 14 mm.	3.59	(17)
Ethyl 4-nitrocinnamate	140	3.50	(17)
Methyl 4-nitro- α -phenylcinnamate	224.5	3.78	(17)
Dimethyl maleate	130	2.55; 2.51	(154); (41)
Dimethyl fumarate	286-287	2.39; 2.23	(154); (41)
Dimethyl citraconate	91	2.68	(42)
Dimethyl mesaconate	202	2.06	(42)
4,4'-Dimethoxyisostilbene†	38	1.77	(150)

* As far as esters are concerned, the melting points refer to the corresponding acids.

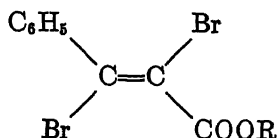
† The isomeric form, m.p. 213°C., was too insoluble to be measured.

of the *cis*-acid (m.p. 159°C.) (which contains phenyl and carboxyl in the *trans*-position with respect to each other) gives mainly *cis*-cinnamic acid, while debromination of the *trans*-acid (m.p. 130°C.) gives *trans*-cinnamic acid; debromination is therefore accompanied by configurational inversion. This may be due to a peculiarity in the substitution of bromine by hydrogen or—more likely—to the fact that no substitution occurs at all, but rather addition of hydrogen to the double bond and subsequent removal of hydrogen bromide (see note 11).

¹⁶ The *trans*-structure of this compound follows from the fact that *o*-amino- α -phenylcinnamic acid, which on deamination gives the isomeric α -phenylcinnamic acid, easily forms a lactam and is therefore the *cis*-compound (158).

Another interesting point may be raised in this connection: The two oximes of the *p*-bromocinnamaldehyde which is obtained (exclusively) upon the addition of hydrobromic acid to phenylpropionaldehyde give with acetic anhydride the nitrile of the β -bromocinnamic acid, m.p. 159°C. (*trans*-acid), and with phosphorus pentachloride the nitrile of the isomeric acid (m.p. 130°C.) (9). It will be interesting to determine the moment of the halogenated aldehyde and so to obtain an insight into the steric mechanism of these dehydration reactions.

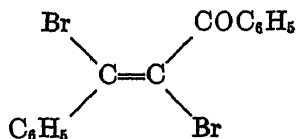
The configuration of the two α, β -dibromocinnamic acids follows from the consideration that the ester



in which the moments of the two bromine atoms cancel each other must have the smaller moment and about the same as that of ethyl cinnamate. This is the ester of the acid melting at 154°C. The isomeric ester, as would be expected, has a higher moment. In this case the same configurations have been assumed previously for other reasons (easier cyclization of the acid melting at 100°C.; other, less conclusive, arguments have been advanced by Stoermer and coworkers (163, 164)). Here, too, the old rule (3, 198) that *trans*-compounds have a weaker absorption than the *cis*-forms is true to the extent that the *cis*-acid is actually yellow.¹⁷

In the formation of the α, β -dibromocinnamic acids from phenylpropionic acid and bromine, *trans*-addition prevails, which is contradictory to a widespread assumption (122).¹⁸

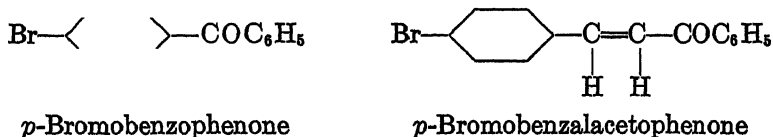
Analogous reasoning permits an evaluation of the dipole moments of the benzalacetophenone derivatives listed in table 3. The dipole moment of α, β -dibromobenzalacetophenone is near enough to that of benzalacetophenone ($\mu = 2.93$) (17) to justify the conclusion that the two bromine moments cancel each other and therefore that the bromine atoms must be in the *trans*-position to each other; here again, in the addition of



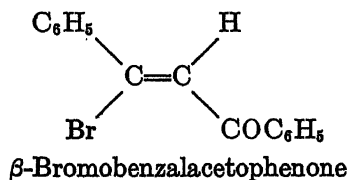
¹⁷ This is by no means generally so. For example, on the basis of this rule Arends (3) has assigned incorrect structures to the α, β -dichloro- and α, β -dibromo-stilbenes.

¹⁸ Ayyar (1) has recently claimed that the free phenylpropionic acid adds bromine in the *cis*-position, while its esters add bromine in the *trans*-position. The first part of this statement is not in accord with our findings.

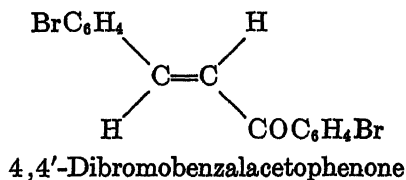
bromine to phenylbenzoylacetylene, the *trans*-reaction is favored. β -Bromobenzalacetophenone and *p*-bromobenzalacetophenone have too different moments for an analogous configuration. As the moment of the latter is close to that of *p*-bromobenzophenone ($\mu = 2.75$) (21), it contains the two polar groups in the *trans*-configuration.



β -Bromobenzalacetophenone, b.p. 219–220°C. at 14 mm, is therefore a *cis*-form,

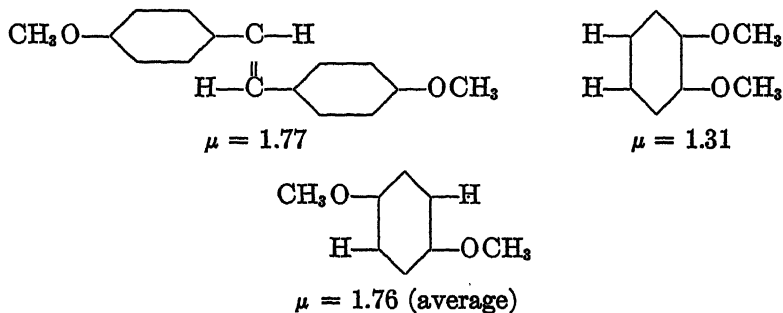


4,4'-Dibromobenzalacetophenone, m.p. 185–186°C., is the *trans*-compound,



as its dipole moment (2.03) is not very different from that of 4,4'-dibromobenzophenone ($\mu = 1.69$) (21).

The dipole moment of the low-melting 4,4'-dimethoxystilbene, on the basis of the same reasoning, would unexpectedly point to the *trans*-configuration, as it is much nearer to that of *p*- than to that of *o*-dimethoxybenzene (93, 95, 191), but this conclusion should await confirmation by determination of the moment of the higher melting isomer, which unfortunately is very slightly soluble in non-polar solvents.



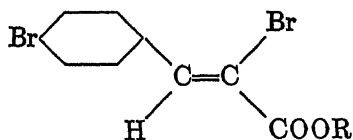
From the inspection of the pairs maleate-fumarate and citraconate-mesaconate it follows that here too, where the theoretical dipole moments are not easily calculated, owing to the interactional forces between the polar groups, the *trans*-compound has the lower dipole moment. This agrees with the fact that diethyl *o*-phthalate has a higher moment (2.6) than diethyl terephthalate (2.2) (72, 193) and will be used later for configurational assignment in a more complicated dicarboxylic acid (see page 578).

In a number of cases, presented in table 4, no conclusions as to configuration can be drawn from the measured dipole moments, as the polar substituents are linked to the same olefinic carbon atom.

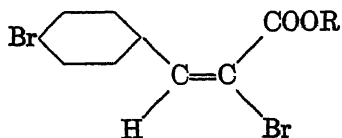
TABLE 4

COMPOUND	PHYSICAL CONSTANTS	μ
4-Bromostilbene.....	M.p. 31°C.; liquid	1.38
		1.38
α -Bromobenzalacetophenone.....	B.p. 218-219°C. at 14 mm.	3.87
Ethyl α -bromocinnamate.....	M.p. (acid) 130-131°C.	2.25
	M.p. (acid) 120°C.	2.80

A decision would be possible if the analogs bearing a polar substituent on the neighboring olefinic carbon atom were prepared according to exactly the same preparative procedure. In this case, assignment of configurational formulas would be possible, and by analogy the structure of the above substances would be made clear. α -Bromocinnamic acid, melting at 130-131°C., is prepared from phenylpropionic acid and gaseous hydrogen bromide in benzene solution. It should be analogous to the isomeric form of 4, α -dibromocinnamic acid, which is obtained from 4-bromopropionic acid and hydrogen bromide under the same conditions:



(expected moment = 2.7)¹⁹



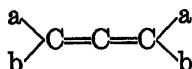
(expected moment = 1.9)¹⁹

In the same way, the form which would be obtained from 4-bromocinnamic acid dibromide and potassium hydroxide solution would be analogous to the α -bromocinnamic acid, m.p. 120°C., which is formed analogously from cinnamic acid dibromide. In a parallel manner, the interesting α -halogenocinnamaldehydes (7) could be studied from the stereochemical viewpoint.

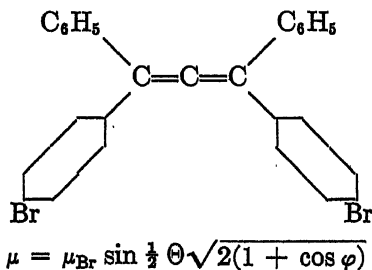
¹⁹ Corresponding to those of the methyl α,β -dibromocinnamates.

The allene system

According to van't Hoff, an allene of the type



should be resolvable into optical antipodes; the two pairs of substituents should lie in two planes perpendicular to each other. The numerous failures to realize optical activity of this kind have eventually led to the suggestion (73) that van't Hoff's prediction is incorrect and that the allene molecule is planar. This general question could be answered by measurements of dipole moments: if in the above formula the two substituents *b*, e.g., are polar, while *a* makes no finite contribution to the electric moment of the molecule, the spatial angle, φ , between the C—*b* valencies and, accordingly, the molecular electric moment, would be different in the diplanar and the planar models. E. Bergmann and Hampson (24) have carried out this investigation, taking into account that the planar model formally would give rise to two configurations, corresponding to a *cis*- and a *trans*-form (angles between the C—*b* vectors 0° and 180° , respectively). After having shown that the tetraphenylallene system in itself has no dipole moment, the authors derived the following equation for the dipole moment of 1,3-di(*p*-bromophenyl)-1,3-diphenylallene



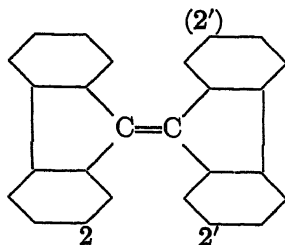
where Θ is the "apparent" angle between the two substituents at the same carbon atom. This has been derived from the dipole moment (1.57) of 1,1-di-*p*-chlorophenyl-3,3-diphenylallene, which is due to two chlorine moments inclined at the angle Θ . The value of approximately 120° , thus found, is in good accord with dipole measurements in the series of benzophenone and 1,1-diphenylethylene (21) and with figures obtained by other physical methods (121, 157, 169).

For the classical model ($\varphi = 90^\circ$) the theoretical value of μ is 1.85, and for the two non-classical possibilities is 0 and 2.62, respectively. The dipole moment actually found was 1.96, so that van't Hoff's model is certainly correct. Subsequently, Maitland and Mills (123), Kohler, Wal-

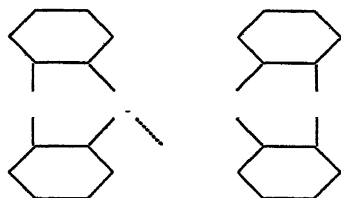
ker, and Tishler (106), and Kohler and Whitcher (107) succeeded in preparing optically active forms of substituted allenes, thus giving final support to the theory of the tetrahedral carbon atom.

2,2'-Difluorodibiphenyleneethylene

A similar problem was encountered in the study of the 2,2'-difluoro derivative of the dark red dibiphenyleneethylene.

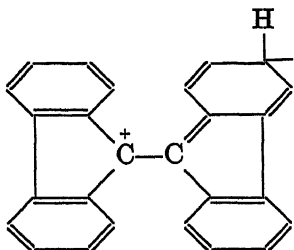


From the orthodox point of view, this substance should exist in two *cis-trans* isomeric forms; but none of the compounds of the fulvenic type has been obtained in geometrically isomeric forms (see, recently, Gleu and Nitzsche (80)), a failure which points to the possibility suggested by Bergmann and Engel (20) that these forms are easily interconvertible through an intermediate step in which the rigidity of the double bond is temporarily cancelled, e.g., through a diradical form (compare Burawoy (45)).

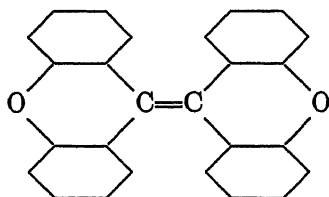


As it seemed interesting to determine the actual configuration of the one isolable form of the above ethylene, its dipole moment was determined. While its *trans*-form should obviously have a zero moment, and its *cis*-form a moment of 2.90, a moment of 2.51 was actually observed (16). The molecule, therefore, would have neither the *cis*- nor the *trans*-configuration, but an intermediate twisted configuration, incompatible with an ethylenic formula at all. One could assume that the molecules (or at least part of them) have constantly a diradical form, but Farkas and Sandler (74; *cf.* 146) were unable to detect paramagnetic properties. It may therefore be that another formula applies, e.g., that of a zwitter ion.²⁰

²⁰ In a discussion in Manchester (November, 1938) Dr. Evans pointed out that these two formulas are not different, but that both represent extreme forms of the same resonance state.

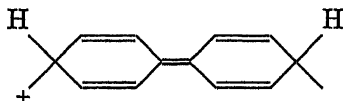


A similar suggestion has been put forward by Schoenberg and coworkers (148, 149) in order to explain the observation that the practically colorless dixanthylene



is converted by heat into a dark blue form. (For "thermochromic ethylenes" in general, see Bergmann and Corte (19)). It will be interesting to study the dipole moments of appropriate representatives of this and similar series (see also page 557).

This position would not be unique, since in the case of diphenyl (and similar substances) analogous conditions prevail. One must assume that other structures besides the classical contribute to the structure of diphenyl, such as



as has been suggested frequently, in order to explain the propagation of effects from one nucleus into the other and to explain the absorption spectra of derivatives of diphenyl (47, 77, 116, 135, 172, 181). A detailed investigation of the spectra of such "fulvenes" as the above would therefore also be of interest.

IV. STEREOISOMERISM IN RING SYSTEMS

As in the case of ethylenes, non-aromatic ring systems bearing two or more substituents give rise to isomerism, and if the substituents are of polar character, the determination of the molecular electric moment enables us to determine the steric arrangement of the substituents. A characteristic case has recently been reported by E. Bergmann and A. Weiz-

mann (33). The addition of chlorine to 1,5-dichloro-, to 9,10-diphenyl-, and to 1,8-dichloro-anthracenes produces dichlorides having dipole moments of 3.7, 3.0, and 2.4, respectively. This means that in the first two cases the halogen adds in the *cis*-position, and in the latter in the *trans*-position (as the moment of 1,8-dichloroanthracene by itself is 3.2, 1,5-dichloro- and 9,10-diphenyl-anthracenes obviously having zero moments). The surprising *cis*-addition is ascribed by the above authors to the possibility that the chlorine molecule as such is able to enter the space between the 9,10-positions, just as oxygen, maleic anhydride, and similar substances do,²¹ while usually halogens add to unsaturated systems by an atomic or ionic mechanism. (For a general review, see 2, 18, 129, 179, 180.)

TABLE 5

SUBSTANCE	MELTING POINT	μ	CONFIGU- RATION	REFERENCE
	°C.			
1,4-Dichlorocyclohexane	102	0	<i>Trans</i>	(84)
1,4-Dibromocyclohexane	111	0	<i>Trans</i>	(84, 94)
1,4-Diiodocyclohexane	67.5	2.4	<i>Cis</i>	(84, 94)
	142	0	<i>Trans</i>	(84, 94)
Dihydrodicyclopentadiene-1,2-dibromide. . .	66	3.20	<i>Cis</i>	(62)
Dihydro- α -tricyclopentadiene-1,2-dibromide..	107	3.28	<i>Cis</i>	(62)
Dihydro- β -tricyclopentadiene-1,2-dibromide..	123	3.26	<i>Cis</i>	(62)
Dihydro- β -tricyclopentadiene-1,2-dibromide..	106	2.06	<i>Trans</i>	(62)
1,2,4,5-Tetrabromocyclohexane	185	2.22		(85)

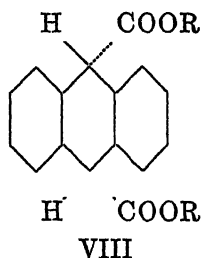
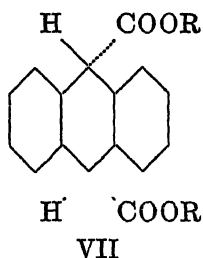
In the preceding section it has been reported that tolane is able to form molecular compounds with the corresponding dihalogen addition products, and for substances interconvertible by the addition or elimination of hydrogen similar effects (formation of mixed crystals) have been known for a long time, provided the substances have a certain geometrical resemblance (44, 66). One of the geometrically isomeric forms of a 1,2-disubstituted ethylene is always more similar to the corresponding acetylene and ethane derivatives. It is therefore interesting that aromatic substances sometimes show an analogous affinity to one of their dihydro derivatives. Haack (83) has observed this for 9-phenyl- and 9,10-diphenyl-anthracenes; even before, it was known that naphthalene and 1,4-dihydronaphthalene give a crystalline molecular compound upon the incomplete reduction of naphthalene (10) or upon the addition of lithium to naphthalene.

Other similar cases of disubstituted isocyclic ring systems are listed in table 5.

²¹ In the case of 1,8-dichloroanthracene the chlorine atoms present may interfere with this mechanism.

The conclusions drawn as to configurations are self-evident. For the 1,2,4,5-tetrabromocyclohexane the dipole moment in itself is not decisive, but it is in accord with the results of an x-ray study of the compound.

In an analogous manner, the moments of substances containing "irregular" substituents (page 554) can be evaluated. The difference in dipole moment between the two dimethyl 9,10-dihydroanthracene-9,10-dicarboxylates (33) (namely, 2.6 and 1.7) points to the *cis*-structure (VIII) for the former (β) (melting point of the acid, 294°C. (with decomposition); melting point of the ester, 163.5–165°C.) and to the *trans*-structure (VII) for the latter (α) (melting point of the acid, 286°C. (with decomposition); melting point of the ester, 162.5–163°C.). This is analogous to the pair maleate-fumarate (page 573), where, however, the dipole moments are not



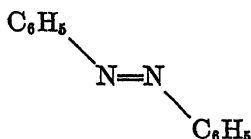
as different as in the above case.

It may finally be mentioned that in an analogous way the configuration of appropriate ethylene oxide derivatives can be determined. Bergmann and Schuetz (28) have compared the two isomeric 4,4'-dinitrostilbene oxides; their moments, 5.75 and 2.1, show that the former compound (m.p. 153–154°C.) has the *cis*-structure (with regard to the relative positions of the nitrophenyl groups), and that the other one (m.p. 200–201°C.) has the *trans*-configuration. In the latter case the moment is expectedly close to that of the unsubstituted ethylene oxide ($\mu = 1.88$) (168) or stilbene oxide ($\mu = 1.73$) (28). In this series, the method is still capable of wider application, as the various 4'-substituted 4-nitrostilbene oxides are easily accessible according to a method worked out by Bergmann and Hervey (25).

V. THE N=N DOUBLE BOND

The case of the azo compounds is completely analogous to that of the ethylenes, azobenzene itself having no dipole moment (23). In view of the fact that the N—C₆H₅ valencies form an angle with the azo bond, and in view of the second observation that 4,4'-dibromoazobenzene and 4,4'-dimethylazobenzene (92) have no finite moment, one must assume that

these substances have the *trans*-configuration.²² The same follows from



the fact that *p*-chloroazobenzene (23), *p*-bromoazobenzene (23), and *p*-methoxyazobenzene (31, 189) have the same dipole moment as the corresponding benzene derivatives. Recently, the *syn*-isomer of azobenzene has been discovered (91) and it has been shown to possess a distinct dipole moment, namely, 3.0 (92). This substance is photochemically reconverted into the stable isomer, and the interconversion can easily be followed by measuring

TABLE 6

SUBSTANCE	μ
Diazoaminobenzene.....	0.90
4,4'-Dichlorodiazoaminobenzene.....	1.94
4,4'-Dibromodiazoaminobenzene.....	1.88
4-Bromodiazoaminobenzene.....	2.00
4-Nitrodiazoaminobenzene.....	4.17
4,4'-Dimethyldiazoaminobenzene.....	0.90
<i>N</i> -Methyldiazoaminobenzene.....	1.48
4,4'-Dibromo- <i>N</i> -methyldiazoaminobenzene.....	2.52

the change in dipole moment with time. (For other physical properties, see Cook, Jones, and Polya (56) and Corrucini and Gilbert (58).) In the case of *trans*-4,4'-dimethylazobenzene and other related compounds, dielectric measurements have shown that light also converts them gradually into the polar *cis*-forms (55).

Le Fèvre and Vine (114) have also made it apparent that the diazoamino compounds possess the *trans*-configuration (table 6).

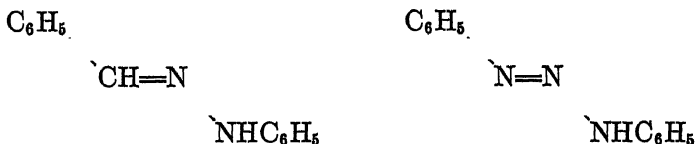
The angle, Θ , at which the two phenyl groups are mutually inclined is about 140° . From the fact that substitution by chlorine in the two 4-positions increases the moment from 0.94 to 1.94, it follows that the two C—Cl vectors (1.55) produce a moment of $1.94 + 0.90$ or of $1.94 - 0.90$. One obtains

$$\begin{array}{ll} (a) \quad 1.94 + 0.90 = 2 \times 1.55 \cos \Theta/2; & \Theta = 47.5^\circ \\ (b) \quad 1.94 - 0.90 = 2 \times 1.55 \cos \Theta/2; & \Theta = 140^\circ \end{array}$$

The former solution, however, is highly improbable and is, moreover, in disagreement with the fact that substitution by two methyl groups in the 4-positions does not affect the moment of diazoaminobenzene.

²² Since stilbene and azobenzene give mixed crystals, stilbene must also be a *trans*-compound as, without experimental proof, has always been tacitly assumed. For the x-ray structure of crystalline stilbene, see reference 138 and compare reference 134.

For diazoaminobenzene the same conclusion has been drawn previously by Ciusa and Pestalozza (54) from the observation that it gives mixed crystals with the *anti*-form of benzaldehyde phenylhydrazone.



Another group of azo compounds to which the method of dipole moments can be applied are the diazocyanides $\text{ArN}=\text{NCN}$ (113) (see table 7). The *trans*-forms in this case should always have the highest moment, with the exception of the nitro compounds, since Le Fèvre and Vine (113) have

TABLE 7

SUBSTANCE	CONFIGURATION	μ
4-Chlorophenyldiazocyanide.....	<i>Cis</i>	2.93
	<i>Trans</i>	3.73
4-Bromophenyldiazocyanide.....	<i>Cis</i>	2.91
	<i>Trans</i>	3.78
2-Bromophenyldiazocyanide.....	<i>Cis</i>	3.79
	<i>Trans</i>	5.32
4-Nitrophenyldiazocyanide.....	<i>Cis</i>	2.04
	<i>Trans</i>	1.47
2,4,6-Tribromophenyldiazocyanide.....	<i>Cis</i>	2.5
	<i>Trans</i>	4.0

shown that the $\text{N}-\text{CN}$ moment will always be directed away from the azo group, as is the case with the $\text{NO}_2-\text{C}_6\text{H}_4-\text{N}$ moment, while the $\text{Hal}-\text{C}_6\text{H}_4-\text{N}$ moment is, although small, directed towards the azo link. The similarity of these substances to the azo compounds proper follows from the observation (156) that their *trans*-forms are also converted photochemically into the *syn*-isomers.

In this series no more fundamentally new discoveries are to be expected; it may only be added that azomethane also has a dipole moment of 0 and therefore has the *trans*-configuration (199), a conclusion which is supported by the investigation of electron diffraction by gaseous azomethane (40).

VI. THE $\text{C}=\text{N}$ DOUBLE BOND

The evaluation of the dipole moments of $\text{C}=\text{N}$ compounds, even the most favorable ones, is hampered by the fact that, in contradistinction to

the C=C and the N=N double bonds, the C=N group in itself possesses a dipole moment. The only very clear-cut case, therefore, in this group, is that of carbodianil (compare 24), $C_6H_5N=C=NC_6H_5$, in which the two C=N moments cancel each other. According to E. Bergmann and W. Schuetz (27), its moment is 1.89 (that of the 4,4'-dimethyl derivative is 1.96). The structure of carbodianil resembles that of tetraphenylallene. Its moment is due to the fact that the two polar bonds (N—C₆H₅) lie in two planes perpendicular to each other and is connected with the con-

TABLE 8*†

SUBSTANCE	μ	REFERENCE
(Benzaldehyde anil)	1.57	(59)
4-Chlorobenzaldehyde anil.	1.77	(59)
4-Chlorobenzaldehyde 4'-methylanil.	2.06	(59)
4-Chlorobenzaldehyde 4'-chloroanil.	1.56	(59)
(Benzophenone anil)	1.96	(21, 86)
(Benzophenone 4''-chloroanil)	2.93	(21, 86)
4-Chlorobenzophenone anil.	1.95	(86)
4-Chlorobenzophenone 4''-chloroanil.	2.49	(86)
(4,4'-Dichlorobenzophenone anil)	0.97	(86)
(Benzophenone chloroimine)	2.96	(175)
(Benzophenone bromoimine)	2.83	(175)
α -4-Chlorobenzophenone chloroimine.	2.47	(175)
β -4-Chlorobenzophenone chloroimine.	2.67	(175)

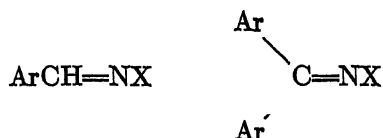
* The complication brought about by the bent nature of the OH and OR groups is so great that salicylideneaniline ($\mu = 2.45$), 5-bromosalicylideneaniline ($\mu = 1.12$), salicylidene-*m*-toluidine ($\mu = 2.59$), and 2-methoxybenzylideneaniline ($\mu = 3.02$) mentioned by de Gaouck and Le Fèvre (59) will not be discussed here and therefore are not included in table 8. The same applies to *p*-homosalicylideneaniline and ethyl salicylidene-*p*-aminobenzoate (60).

† The substances in brackets either gave no stereoisomeric forms, or their structure cannot be defined by evaluation of the dipole moment.

figuration of the molecule by the same formula as for tetraphenylallene (page 574). Assuming the N—C₆H₅ moment to be 1.34 (173), and that the angle between the C=N and the N—C₆H₅ bond is 120°,—corresponding to the valency angle in the allene molecule,—the moment of carbodianil should be 1.64. The difference may be due to inexactness of the assumptions made.

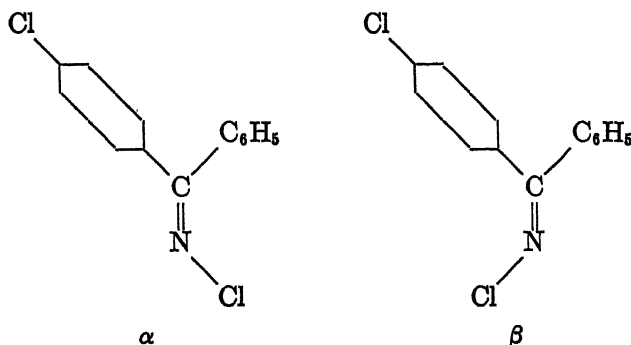
Benzalazine, $C_6H_5CH=N-N=CHC_6H_5$, also seems to be a simple case, but, as has been pointed out by Sutton (173), three *trans*-structures are possible, only one of them being polar. The significance of the slight dipole moment (0.89 (96); 1.00, 1.04 (182)) is therefore uncertain.

A second, less simple case is that of the benzaldehyde imines and benzophenone imines:

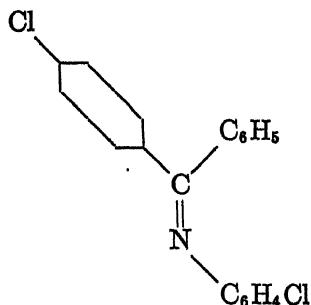


The experimental material available is summarized in table 8.

The difference in moment between the last two substances, although small, is most probably due to different configurations, the α -compound having the chlorine atoms on different sides relative to the C=N plane.



The smallness of the difference is due to the smallness of the nitrogen-halogen moments, as pointed out by Theilacker and Fauser (175). It is further obvious that 4-chlorobenzophenone 4'-chloroanil must also be the *trans*-compound (corresponding to α),



as its moment is identical with that of α -4-chlorobenzophenone chloroimine. That, as would be expected, the interposition of the phenyl between nitrogen and chlorine is of no influence, follows also from the identity in the moments of benzophenone 4'-chloroanil and benzophenone chloroimine.

Since in the other cases no stereoisomeric forms are known, the assignment of a configurational formula is more difficult. If ν is the value of the $\text{C}_6\text{H}_5\text{—N}$ moment, and α the angle between ν and the moment ξ of the C=N bond, then these data are connected with the moment, μ , of benzophenone anil by the formula

$$\mu^2 = \nu^2 + \xi^2 - 2\nu\xi \cos (180 - \alpha)$$

If we assume ν to be -1.34 and α to be 120° , we obtain

$$\mu^2 = 1.34^2 + \xi^2 - 1.34\xi$$

and with $\mu = 1.97$, $\xi = 2.27$.

Following a calculation made by Theilacker and Fauser, the moment of the *trans*- and the *cis*-forms (y, z) of 4-chlorobenzophenone anil would then be given by the following formulas:

$$y^2 = \mu_{\text{Cl}}^2 + \xi^2 + \nu^2 - \xi\mu_{\text{Cl}} + \nu\xi - 2\nu\mu_{\text{Cl}}$$

$$z^2 = \mu_{\text{Cl}}^2 + \xi^2 + \nu^2 - \xi\mu_{\text{Cl}} + \nu\xi + \nu\mu_{\text{Cl}}$$

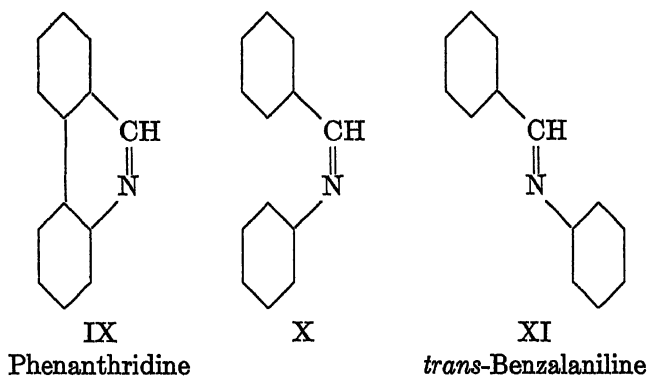
$$y = 2.6; z = 0.9$$

4-Chlorobenzophenone anil ($\mu = 1.95$) is therefore the *trans*-compound. The *trans*-compound should have the higher moment, as the direction of the $\text{N—C}_6\text{H}_5$ moment is not opposite to that of the C—Cl bond.

With regard to the benzaldehyde derivatives, it is difficult to understand at first glance why they have so much lower dipole moments than the corresponding benzophenone derivatives, and one would come to think that they all are *cis*(*syn*)-compounds. As, however, the dipole moments of benzylideneaniline and of 4-chlorobenzylidene-4'-chloroaniline are essentially identical, one must assume that both these substances are *trans*-compounds, and the same will apply to other representatives of this series.

The apparent discrepancy between the anils of benzophenone and benzaldehyde may be explained by the assumption that the valency angles at the doubly linked carbon atom are not identical in both cases, the distribution in the case of two phenyl substituents being probably more regular. A similar reasoning may apply to the recent observation of de Gaouck and Le Fèvre (60) that phenanthridine (IX), which may be regarded as analogous to the unknown *cis*-benzalaniline (X), has in fact the same dipole moment as *trans*-benzalaniline (XI).

It may be recalled in this connection that, in general, the Raman frequencies of the C=N double bond coincide with those of the *trans*-ethylenic system.

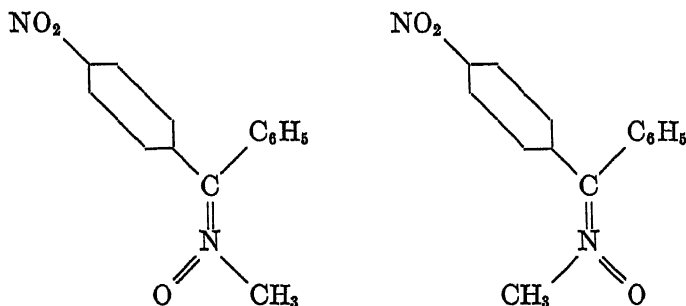


A still more complicated case is that of the oximes and phenylhydrazones, where by the oxygen and the imino nitrogen a new factor of uncertainty is introduced into the calculation. As a matter of fact, only the comparison of isomeric pairs can lead to success here and, in the hands of Sutton and Taylor (174), has already done so (table 9).

TABLE 9

SUBSTANCE	μ
4-Nitrobenzophenone α -oxime <i>N</i> -methyl ether.....	6.60
4-Nitrobenzophenone β -oxime <i>N</i> -methyl ether.....	1.09
4-Nitrobenzophenone α -oxime <i>O</i> -methyl ether.....	3.75
4-Nitrobenzophenone β -oxime <i>O</i> -methyl ether.....	4.26
4-Nitrobenzaldehyde β -oxime <i>N</i> -methyl ether.....	6.40
4-Nitrobenzaldehyde α -oxime <i>O</i> -methyl ether.....	3.40
4-Nitrobenzaldehyde β -oxime <i>O</i> -methyl ether.....	3.89

It becomes clear at once that the α - and β -compounds in both series correspond to each other, and an inspection of the formulas for the *N*-methyl ethers shows that the compound with the low moment must have



the —NO_2 and the =O groups on different sides of the C=N double bond.

For the *O*-methyl ethers, the correlation between dipole moment and the molecular architecture cannot be derived, for reasons given above. But as for the 4-nitrobenzophenone oximes the configuration is known through the *N*-methyl ethers, and the same applies to the *O*-methyl compounds, so that the parallelism between the conditions in the benzophenone and the benzaldehyde series is a sufficient indication as to the structure of the isomeric benzaldoximes. How difficult the conditions may become is evident from the moments observed by Parsons and Porter (133) for the isomeric oximes of the three chlorobenzophenones (table 10). It would be expected that—at least in the para series—the difference would be as great as for the 4-nitrobenzophenone oxime *O*-methyl ethers and would lie in the same direction, but apparently the presence of the free hydroxyl groups, which would cause association (and in the different cases to a different extent), effaces the influence of the configurational factors.

Nevertheless, the experiments of Sutton and Taylor have shown that measurements of dipole moments will give a valuable lead towards elucidating the configurational relationships in the oxime series and they have given final support to Meisenheimer's (127) thesis that in the Beckmann rearrangement of oximes exchange of *trans*-substituents takes place, e.g.,

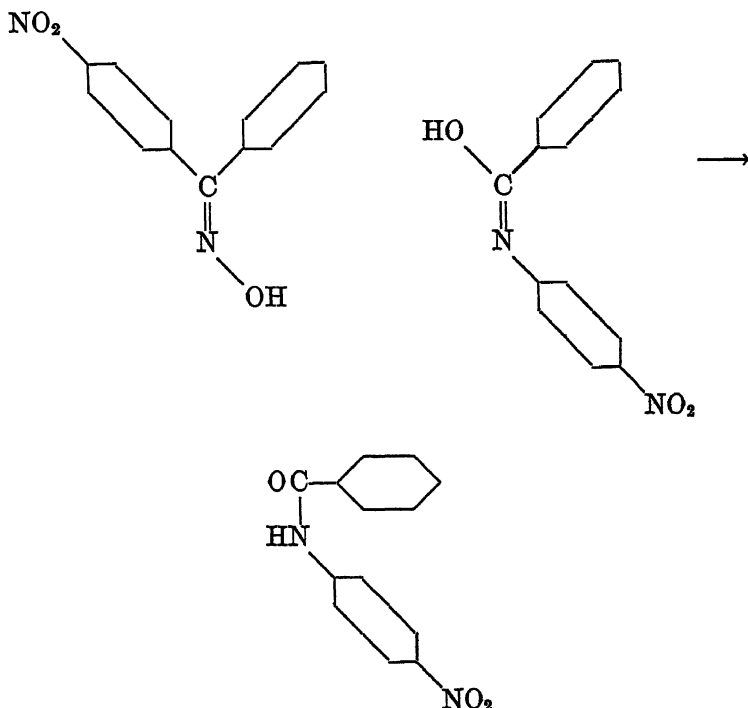
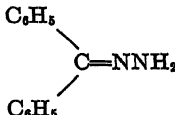
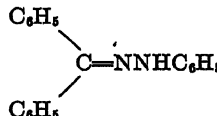
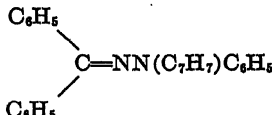
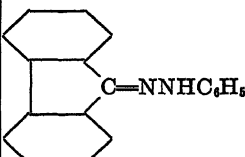
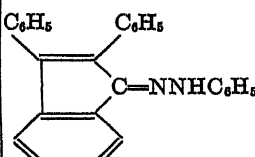


TABLE 10

SUBSTANCE

α -2-Chlorobenzophenone oxime.	1.61
β -2-Chlorobenzophenone oxime.	1.61
α -3-Chlorobenzophenone oxime.	1.50
β -3-Chlorobenzophenone oxime.	1.61
α -4-Chlorobenzophenone oxime.	2.32
β -4-Chlorobenzophenone oxime.	2.38

TABLE 11

SUBSTANCE	FORMULA	μ	REFERENCE
Benzophenone hydrazone.....		2.02	(32)
Benzophenone phenylhydrazone.....		2.22	(32)
Benzophenone benzylphenylhydrazone		2.60	(32)
Fluorenone phenylhydrazone.....		2.12	(32)
2,3-Diphenylindone phenylhydrazone.		1.93	(32)
Benzaldehyde phenylhydrazone.....	$\text{C}_6\text{H}_5\text{CH}=\text{NNHC}_6\text{H}_5$	2.06	(182)
α -Nitroformaldehyde phenylhydrazone	$\text{NO}_2\text{CH}=\text{NNHC}_6\text{H}_5$	3.34	(102)
α -Acetaldehyde phenylhydrazone.....	$\text{CH}_3\text{CH}=\text{NNHC}_6\text{H}_5$	2.61	(102)
β -Acetaldehyde phenylhydrazone.....		2.58	(102)
Acetone phenylhydrazone.....	$(\text{CH}_3)_2\text{C}=\text{NNHC}_6\text{H}_5$	2.68	(102)
α -Benzoylformaldehyde phenylhydrazone.....	$\text{C}_6\text{H}_5\text{COCH}=\text{NNHC}_6\text{H}_5$	1.70	(102)
β -Benzoylformaldehyde phenylhydrazone.....		2.72	(102)

It would therefore be interesting to use this method also for the oximes of substituted benzalacetophenones and cinnamaldehydes. A discussion of the prevailing relationships by Auwers and coworkers from one side (5, 6, 8) and Blatt from the other side (37, 38) has brought no final decision, which is all the more to be desired, as, on the basis of the pertaining facts, Auwers still raises some doubt as to the general validity of Meisenheimer's theory.

TABLE 12

c	ρ	ϵ	n^2	$P_{\frac{1}{2}}$	$P_{E\frac{1}{2}}$	P	P_E	P_{A+O}	P_{A+O}^∞	μ
4,4'-Dimethoxyisostilbene; $t = 16.0^\circ\text{C}.$; in benzene										
0	0.8834	2.2920	2.2920	26.579	26.579			→		
0.01415	0.8911	2.3733	2.3106	28.294	27.395	147.81	84.23	63.58		
0.01917	0.8938	2.4014	2.3178	28.891	27.694	147.16	84.72	62.44	66.9	1.77
0.02393	0.8964	2.4262	2.3239	28.430	27.966	145.73	84.51	61.22		
0.02792	0.8986	2.4507	2.3287	29.933	28.188	146.70	84.18	62.52		
4-Aminodiphenyl; $t = 21.8^\circ\text{C}.$; in benzene										
0	0.8765	2.2803	2.2803	26.618	26.618			→		
0.01848	0.8830	2.3774	2.3037	28.396	27.341	122.87	63.18	59.69		
0.02819	0.8864	2.4290	2.3106	29.327	27.636	122.72	62.73	59.99	61.0	1.71
0.03418	0.8884	2.4554	2.3179	29.382	27.865	120.36	63.13	57.23		
0.04504	0.8932	2.5192	2.3316	30.933	28.287	122.43	63.68	58.75		
2-Bromo-6-nitronaphthalene; $t = 20.4^\circ\text{C}.$; in benzene										
0	0.8782	2.2817	2.2817	26.588	26.588			→		
0.00352	0.8831	2.3078	2.2866	27.312	26.719	232.14	63.82	168.32		
0.00490	0.8850	2.3468	2.2883	27.606	26.767	234.48	63.20	171.28	172.7	2.87
0.00597	0.8865	2.3632	2.2892	27.854	26.798	238.81	61.75	174.06		
0.00730	0.8884	2.3608		28.124		236.95		174.03		
2-Nitro-3,5-dimethylbromobenzene; $t = 23.0^\circ\text{C}.$										
0	0.8751	2.2779	2.2430	26.626	26.111			→		
0.00287	0.8789	2.3691	2.2440	27.969	26.162	494.57	44.02	450.55		
0.00626	0.8833	2.4337	2.2464	28.912	26.244	391.90	47.43	344.47	334.2	4.00
0.00393	0.8802	2.3688		27.978		371.25	(47.43)	323.82	(average)	

In table 11 a number of phenylhydrazones have been listed; some of them have been discussed by Jensen and Bak (102) without any final decision as to their configuration.

VII. APPENDIX

In this appendix are reproduced a few dipole moment data which have not been published previously and to which reference has been made in the preceding pages.

A. Substances

4,4'-Dimethoxyisostilbene (m.p. 35°C.) was prepared according to the method given by Schlenk and Bergmann (145). Prepared according to Schlenk (143), 4-aminodiphenyl melts at 51°C. For 2-nitro-3,5-dimethylbromobenzene, the data are given in note 2, while 2-bromo-6-nitronaphthalene was obtained by the following procedure: 13 g. of 6-nitro-2-naphthylamine (1) was dissolved in concentrated sulfuric acid, the solution was poured into ice (80 g.) and was then diazotized with sodium nitrite (5 g.). To this solution a warm (40°C.) solution of sodium bromocuprite was added (from 27 g. of copper sulfate, 75 g. of sodium bromide, and 7 g. of copper bronze in 200 cc. of water), and the solid reaction product was filtered off, dried, and thoroughly extracted with ether. The ether residue was first distilled *in vacuo* and was then recrystallized from glacial acetic acid. The yield was 10 per cent, and the substance melted at 191°C. (Calculated for $C_{10}H_6O_2NBr$: C, 47.6 per cent; H, 2.4 per cent. Found: C, 47.8 per cent; H, 2.6 per cent.)

B. Measurements

The figures in table 12 have the following significance: c , molar fraction; ρ , density; ϵ , dielectric constant; n , refractive index; P_t , total polarization (P_{E_t} , electronic polarization) for the solution; $P(P_E)$ the same for the solute; P_{A+O} , atomic and orientation polarization, which is extrapolated graphically to infinite dilution (P_{A+O}^∞).

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